National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-55568-8

Canada

THE UNIVERSITY OF ALBERTA

THE EFFECTS OF ASCORBIC ACID AND PECTIN ON THE LIPID STATUS OF HIGH CHOLESTEROL-FED MICE

BY

SANDRA REGINA PETRIZ DE ASSIS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN NUTRITION

DEPARTMENT OF FOODS AND NUTRITION

EDMONTON, ALBERTA, CANADA

FALL 1989

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: SANDRA REGINA PETRIZ DE ASSIS

TITLE OF THESIS: THE EFFECTS OF ASCORBIC ACID AND PECTIN ON THE LIPID STATUS OF HIGH CHOLESTEROL-FED MICE

DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE WAS GRANTED: FALL 1989

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

HIGS 705-bloco D-casa 74

70350-Brasilia/DF-BRAZIL

Date: June 21, 1989.

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "THE EFFECTS OF ASCORBIC ACID AND PECTIN ON THE LIPID STATUS IN HIGH CHOLESTEROL-FED MICE" submitted by SANDRA REGINA PETRIZ DE ASSIS in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in NUTRITION.

T. Basu — Supervisor

M. Gee

M. McBurney

S. Yokoyama — External Examiner

DATE: May 31, 1989.

To my loving family: my husband André and our sons Bernardo and Daniel.

ABSTRACT

This study was undertaken to investigate the short and long-term effects of pectin (5%) and ascorbic acid (1 mg/ml drinking water), either alone or in combination, on lipid status in mice receiving a semi-synthetic diet containing 1% cholesterol. Feeding the pectin diet ad libitum to mice for 4 weeks resulted in significantly lower plasma levels of total cholesterol (T-C), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C), as well as, hepatic levels of T-C than those of the animals receiving a diet containing 1% cholesterol alone. The magnitude of reductions in plasma T-C and LDL-C was even more pronounced when the pectin containing high cholesterol diet was fed for 12 weeks. Plasma high-density lipoprotein cholesterol (HDL-C) was found to be reduced while the HDL-C/LDL-C ratio was increased, in animals receiving pectin for 12 weeks. Similar changes in plasma lipid levels were observed in animals receiving a high cholesterol diet containing 5% pectin, but isocalorically, for 8 weeks. Furthermore, fecal cholesterol concentrations of these animals were similar to those receiving isocalorically a 1% cholesterol diet without pectin. These results indicate that the pectin-associated changes in lipid profile were not the reflection of food intake or lipid absorption.

Daily administration of ascorbic acid to high cholesterol fed animals for 12 weeks resulted in

significantly lower plasma T-C and HDL-C concentrations than those of the high cholesterol-fed control animals. The plasma LDL-C and TG concentrations, however, remained unaffected by ascorbic acid supplementation. Interestingly enough, the vitamin supplementation for 12 weeks resulted in a marked elevation in the hepatic concentration of cholesterol. The histological examination of the liver of these animals also revealed excessive lipid droplets in hepatocytes. Feeding pectin concomitantly with ascorbic acid showed no synergistic effect in lowering lipid status. The hypolipidemic effect observed in this study was essentially attributed to pectin alone.

The pectin associated reduction in cholesterol status is clearly evident in mice. This effect does not seem to be the reflection of total food intake or absorption. Since this species does synthesize ascorbic acid endogenously, the true effect of ascorbic acid cannot be determined until this study is carried out in species such as guinea pigs, which are unable to make the vitamin in their body.

ACKNOWLEDGEMENT

This thesis was possible because the love and cooperation of my family: my husband André for his endless encouragement, support and dedication and our sons Bernardo and Daniel for their affection and understanding. To them I express my profound thanks.

I wish to thank my supervisor Dr. Tapan K. Basu for his continuous assistance and guidance provided during this research.

The financial support provided by the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (Brazil), is gratefully acknowledged.

I owe special thanks to Ronaldo D. Ferreira for his valuable help with the animal care; Chris Lin and Dr. Y. Goh who have given so generously of their time and advice during the laboratory work; Sérgio da Fonseca for his help with the statistical analysis; Antônio Carlos Joussef for the biochemical advice; Dr. H. Idikio from the Department of Pathology of the University of Alberta Hospital for the histological analysis of the liver samples and to all my other friends who have encourageded me during this work.

I would like to express my gratitude to my parents Thaís and João Petriz for their love, dedication and support throughout my life.

TABLE OF CONTENTS

1. INTRODUCTION 01
1.1. CHOLESTEROL METABOLISM 01
1.1.1. SYNTHESIS 01
1.1.2. TRANSPORT 02
1.1.3. CATABOLISM 05
1.2. CHOLESTEROL RELATED DISEASE 08
1.3. DRUGS CONTROLLING BLOOD CHOLESTEROL 09
1.4. NUTRITIONAL FACTORS AFFECTING CHOLESTEROL METABOLISM 11
1.4.1. CHOLESTEROL, SATURATED FATTY ACIDS AND TOTAL CALORIC INTAKE 12
1.4.2. POLYUNSATURATED FATTY ACIDS 14
1.4.3. DIETARY FIBER 15
1.4.4. VITAMIN C 17
1.5. CONCLUSION AND OBJECTIVES OF THE PRESENT STUDY. 23
2. METHODOLOGY 28
2.1. ANIMALS AND DIETS 28
2.1.1. STUDY 1: SHORT-TERM EFFECTS 28
2.1.2. STUDY 2: LONG-TERM EFFECTS 31
2.1.3. PAIR-FED CONTROL STUDY 31
2.2 ANALYTICAL METHODS 32
2.2.1. DETERMINATION OF PLASMA TOTAL CHOLESTEROL 33
2.2.2. DETERMINATION OF PLASMA HDL CHOLESTEROL 34

2.2.3. DETERMINATION OF LDL CHOLESTEROL 35
2.2.4. DETERMINATION OF TRIGLYCERIDES 36
2.2.5. DETERMINATION OF THE LIVER TOTAL CHOLESTEROL 38
2.2.6. DETERMINATION OF FECAL CHOLESTEROL 40
2.2.7. DETERMINATION OF HEPATIC CONCENTRATION OF ASCORBIC ACID (VITAMIN C) 41
2.3. STATISTICAL ANALYSIS 41
2.4. HISTOLOGICAL ANALYSIS 43
3. EXPERIMENTAL RESULTS 44
3.1. EFFECT OF HIGH CHOLESTEROL DIET (1%) ON THE LIPID STATUS IN MICE 44
3.2. EFFECT OF PECTIN (5%) ON THE LIPID STATUS IN HIGH CHOLESTEROL (1%) FED MICE 47
3.3. EFFECT OF ASCORBIC ACID ON THE LIPID STATUS IN HIGH CHOLESTEROL FED MICE..... 60
3.4. EFFECT OF PECTIN IN COMBINATION WITH ASCORBIC ACID ON THE LIPID STATUS IN HIGH CHOLESTEROL FED MICE 68
4. DISCUSSION 77
4.1. CONCLUSION 85
REFERENCES 88

LIST OF TABLES

TABLE PAGE

2.1 Diet Composition 29

2.2 Experimental groups and their respective diets 30

3.1 Body weight gain, and the liver weight relative to body weight in mice fed a high cholesterol (H-C) diet for 4 and 12 weeks 45

3.2 Plasma lipid profiles in mice fed a high cholesterol (1%) diet for 4 and 12 Weeks 48

3.3 Hepatic cholesterol levels in mice fed a high cholesterol (1%) diet for 4 and 12 weeks 49

3.4 Effect of pectin (Pe) on the body weight gain and liver weight relative to body weight in high cholesterol (1%) fed mice for 4 and 12 weeks 51

3.5 Plasma lipid profiles in mice fed a high cholesterol (1%) diet with (5%) and without pectin for 4 weeks . 53

3.6 Hepatic cholesterol levels in mice fed a high cholesterol (1%) diet with (5%) and without pectin for 4 weeks 54

3.7 Plasma lipid profiles in mice fed a high cholesterol (1%) diet with (5%) and without pectin for 12 weeks 55

3.8 Hepatic cholesterol levels in mice fed a high cholesterol diet (1%) with (5%) and without pectin for 12 weeks 57

3.9 Plasma lipid profiles in mice fed an isocaloric high

cholesterol (1%) diet with (5%) and without pectin for 8 weeks 59

3.10 Fecal weight and cholesterol concentration in mice fed an isocaloric high cholesterol diet with (5%) and without pectin for 4 weeks 61

3.11 Effect of ascorbic acid (AA) on the body and liver weights in high cholesterol (1%) fed mice for 4 and 12 weeks 62

3.12 Effect of ascorbic acid on plasma lipid profiles in high cholesterol (1%) fed mice for 4 and 12 weeks .. 63

3.13 Effect of ascorbic acid on the hepatic cholesterol concentration in high cholesterol (1%) fed mice for 4 and 12 weeks 65

3.14 Plasma lipid profiles in mice fed an isocaloric high cholesterol (1%) diet either alone or with ascorbic acid for 8 weeks 67

3.15 Fecal weight and cholesterol concentration in mice fed an isocaloric high cholesterol diet either alone or with ascorbic acid for 4 weeks 69

3.16 Effect of pectin and ascorbic acid either alone or in combination on the body and liver weights in high cholesterol (1%) fed mice for 4 and 12 weeks .. 70

3.17 Effect of pectin and ascorbic acid either alone or in combination on plasma lipid profiles in mice fed a high cholesterol (1%) diet for 4 weeks 71

3.18 Effect of pectin and ascorbic acid either alone or in combination on plasma lipid profiles in mice fed

a high cholesterol (1%) diet for 12 weeks 73

3.19 Effect of pectin and ascorbic acid either alone or in combination on the hepatic cholesterol concentration in high cholesterol (1%) fed mice for 4 weeks 74

3.20 Effect of pectin and ascorbic acid either alone or in combination on the hepatic cholesterol concentration in high cholesterol (1%) fed mice for 12 weeks 75

LIST OF FIGURES

FIGURE PAGE

1.1 Transport of cholesterol via lipoproteins (modified after Grundy, 1986) 03

1.2 Schematic presentation of the bile acid metabolism (modified after Mayes, 1988) 06

1.3 Effect of a diet high in saturated fats, cholesterol and calories (obesity) on the metabolism of lipoproteins (modified after Grundy, 1986) 13

1.4 Microsomal hydroxylating enzyme system and cytochrome P450 (modified after Basu and Schorah, 1982) 20

1.5 Proposed mechanism of a synergistic effect between ascorbic acid and pectin in lowering cholesterol levels (modified after Ginter et al., 1982b) 25

2.1 Standard curve for cholesterol determination 39

2.2 Standard curve for ascorbic acid determination 42

LIST OF PLATES

PLATE PAGE

3.1 Photographs of the livers from animals; (A) fed a normal diet (control A); (B) fed a 1% cholesterol diet (control B) 46

3.2 Photomicrographs of the livers from animals; (A) fed a control diet (control A) for 12 weeks (magnification 10X); (B) fed a 1% cholesterol diet (control B) for 12 weeks (magnification 25X) 50

3.3 Photomicrograph of the liver from an animal fed a 1% cholesterol diet with pectin (5%) for 12 weeks (magnification 10X) 58

3.4 Photomicrograph of the liver from an animal fed a 1% cholesterol diet with ascorbic acid supplemented (1 mg/ml) in drinking water for 12 weeks (magnification 25X) 66

3.5 Photomicrograph of the liver from an animal fed a 1% cholesterol diet with pectin (5%) and ascorbic acid supplemented (1 mg/ml) in drinking water for 12 weeks (magnification 25X) 76

ABBREVIATIONS

AA	... ascorbic acid
ACAT	... acyl CoA-cholesterol acyl transferase
C	... concentration
^{o}C	... degree Celsius
dl	... deciliter
EPA	... eicosapentaenoic acid
FCR	... fractional catabolic rate
g	... gram
H-C	... high cholesterol
HDL	... high-density lipoprotein
HDL-C	... high-density lipoprotein cholesterol
HMG CoA	... 3-hydroxy - 3 methyl glutaryl - CoA
kg	... kilogram
l	... liter
LCAT	... lecithin cholesterol acyl transferase
LDL	... low-density lipoprotein
LDL-C	... low-density lipoprotein cholesterol
LSD	... least significant difference
mg	... milligram
min	... minute
ml	... milliliter
mmol	... millimole
nm	... nanometer
Pe	... pectin
RNI	... recommended nutrient intake

rpm	... rotations per minute
SEM	... standard error of the mean
T-C	... total cholesterol
TG	... triglycerides
VLDL	... very low-density lipoprotein
VLDL-C	... very low-density lipoprotein cholesterol
v/v	... volume per volume
w/v	... weight per volume
umol	... micromole

CHAPTER 1. INTRODUCTION

1.1. CHOLESTEROL METABOLISM

Cholesterol is a lipid (steroid) essential for the body which serves as structural component of cell membranes. Its derivatives, such as bile acid, steroid hormones and vitamin D play an important role in the body (Ginter et al., 1982a; McMurray, 1983 and Mayes, 1988).

1.1.1. SYNTHESIS

Cholesterol in mammals is mainly synthesized in the liver (approximately 50%) and gut (approximately 15%), but it can also be synthesized in the skin and to a lesser extent in almost all other tissues (Mayes, 1988 and Guyton, 1986).

In humans the endogenous cholesterol is synthesized from acetate. Initially two molecules of acetyl-CoA condense to form acetoacetyl-CoA. The 3-hydroxy-3methyl-glutaryl CoA (HMG CoA) is formed from the reaction of acetoacetyl-CoA with a further acetyl-CoA molecule. The intermediate HMG CoA is reduced to mevalonic acid in the presence of $NADPH.H^+$ and it is catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase (Fidanza et al., 1982). The HMG CoA pathway is considered to be quantitatively the most important in cholesterol

biosynthesis (Mayes, 1988). Cholesterol is subsequently formed through several steps via mevalonic acid and squalene (Fidanza et al., 1982). The cholesterol regulation in the liver occurs by a feedback mechanism, which is mediated by changes in the activity of HMG-CoA reductase; the key enzyme in cholesterol synthesis (Stryer, 1981). Dietary cholesterol inhibits the HMG-CoA reductase in the liver and can inactivate the existing enzyme molecule.

1.1.2. TRANSPORT

The cholesterol transport via lipoproteins is outlined in Fig. 1.1. Exogenous cholesterol is absorbed from the intestine and it is incorporated into chylomicrons. Chylomicron remnants originated from the hydrolysis of triglycerides of chylomicrons are removed by the liver. Very low-density lipoproteins (VLDL) are secreted by the liver and then degraded into VLDL remnants, which can be removed by the liver or be transformed to LDL (Grundy, 1986 and Vega and Grundy, 1987). Approximately two-thirds of the cholesterol in the blood is found in LDL which is the major cholesterol-carrying lipoprotein in the plasma (McGilvery, 1983). Most of the LDLs carrying cholesterol in the form of cholesteryl esters can be removed directly from the circulation by LDL receptors on the liver cells and a smaller fraction is taken up by extrahepatic tissues (Goldstein and Brown, 1984 and Grundy, 1986).

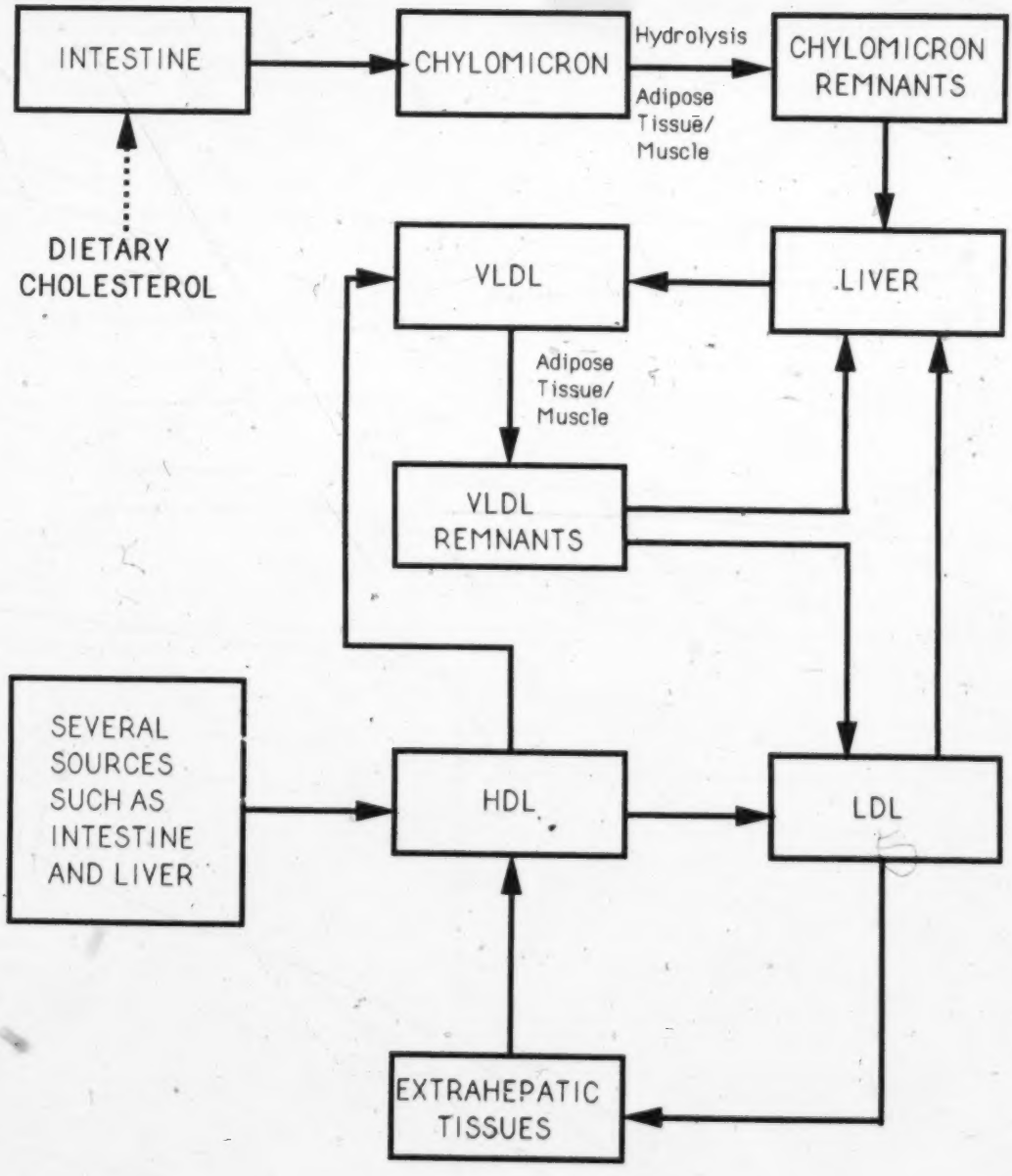

Figure 1.1 Transport of cholesterol via lipoprotein (modified after Grundy, 1986)

In the cells the LDL binds to specific receptors on the plasma membrane. The LDL-receptor complex is incorporated into the cell by endocytosis. Vesicles containing LDL fuse with lysosomes, and the cholesteryl ester is hydrolyzed by lysosomal lipases to free fatty acids and free cholesterol. In all cells, cholesterol is incorporated in newly synthesized surface membranes, however, in some specialized cells cholesterol can be converted to steroid hormones (adrenal gland and ovary) and bile acids (liver). Excessive cholesterol within the cell is esterified in the Golgi apparatus into cholesteryl oleate by acyl CoA-cholesterol acyl transferase (ACAT). It suppresses the synthesis of endogenous cholesterol by reducing HMG CoA reductase activity and also inhibits the production of LDL receptors (Brown and Goldstein, 1984).

High-density lipoproteins (HDL) are synthesized by the liver and gut. It appears to take up cholesterol from extra hepatic tissues back to the liver, consequently preventing its accumulation on those tissues. This process has been called "reverse cholesterol transport". The free cholesterol on HDL is esterified by the enzyme lecithin cholesterol acyltransferase (LCAT) to cholesteryl ester. The latter can be transferred to VLDLs and LDLs, and consequently be removed by the liver (Fidanza et al., 1982; Stryer, 1981; McGilvery, 1983; Grundy, 1986 and Mayes, 1988).

1.1.3. CATABOLISM

Bile acids are the principal products of the cholesterol catabolism, which takes place in the liver cells (Mayes, 1988). About 30-60% of the daily cholesterol production is converted to bile acids, being those delivered into the small intestine in the bile secretions (Linscheer and Vergroesen, 1988) (Fig. 1.2, A).

In man, cholic and chenodeoxycholic acid are the primary bile acids, being synthesized in the liver directly from cholesterol. They undergo changes in the gut by the intestinal flora, forming deoxycholic and lithocholic acids, the secondary bile acids (Mayes, 1988).

Approximately 98% of the bile acids are reabsorbed by the gut mucosa (ileum) and then returned to the liver via the portal vein, a process called "enterohepatic circulation" (Mayes, 1988) (Fig. 1.2, B). The small amount of bile acids not reabsorbed are eliminated in feces (Einarssom and Angelin, 1986) (Fig. 1.2, C).

The formation of bile acids is believed to be under a negative-feedback control by the bile acids reabsorbed in the gut (Mayes, 1988) (Fig. 1.2, D). The rate-limiting reaction in the pathway for bile acids synthesis is the 7α-hydroxylation of cholesterol, which is catalyzed by the enzyme 7α-hydroxylase (Linscheer and Vergroesen, 1988). This enzyme is located in the liver microsomes and belongs to a group called "mixed-function oxygenase", which

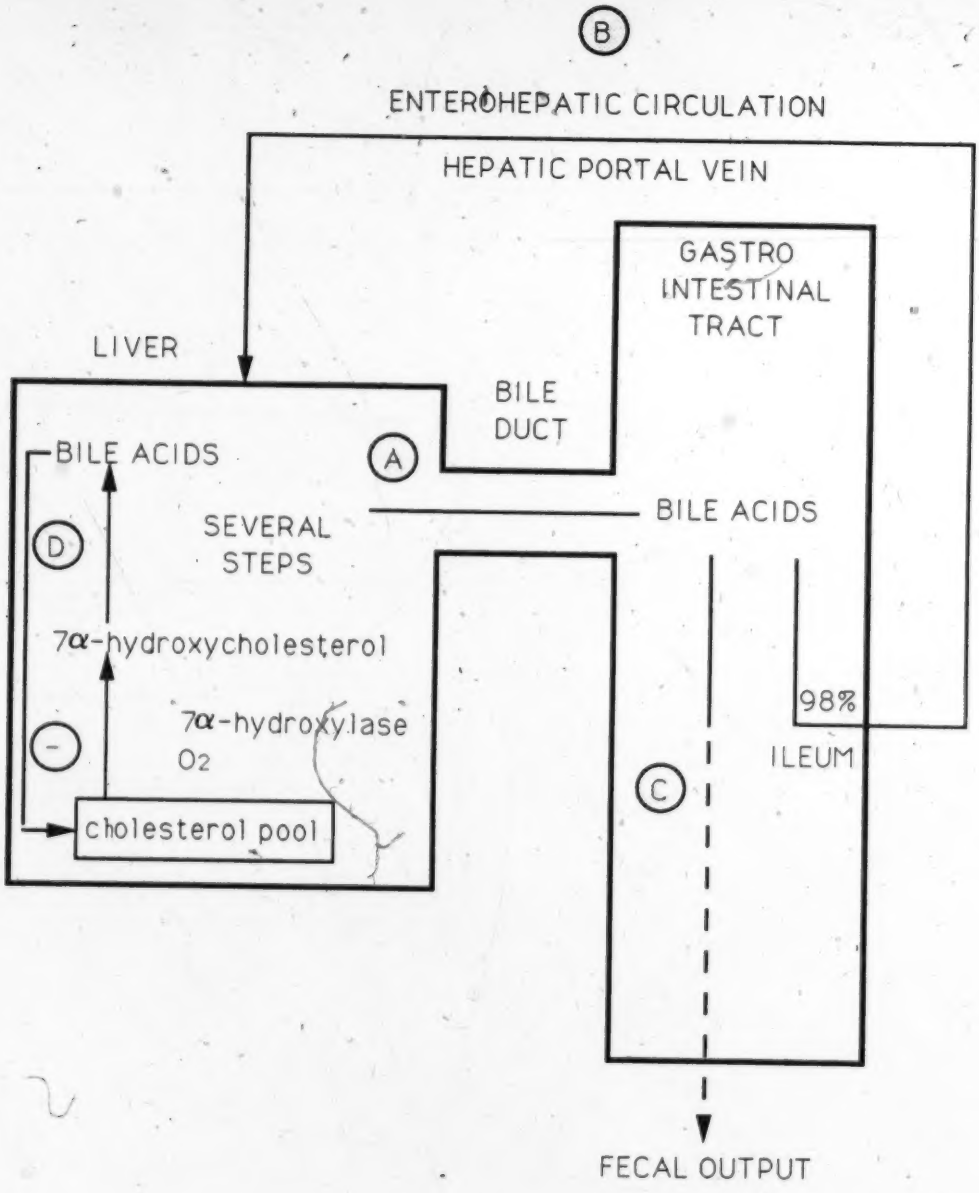

Figure 1.2 Schematic presentation of the bile acid metabolism Circular letters A-D, see text (modified after Mayes, 1988).

requires several components such as demethylating substrates, flavoproteins, a cytochrome P450 hemo protein, oxygen and NADPH (Fidanza et al., 1982 and Basu and Schorah, 1982). Bile acids can inhibit the 7α-hydroxylase, however the exact mechanism is not clear (Mayes, 1988).

The enterohepatic circulation of bile acids is very important to the control of the body's cholesterol pool. Normally, a constant pool of bile acids is maintained since an equivalent amount to that lost in the feces is synthesized in the liver (Fidanza et al., 1982 and Mayes, 1988). If the enterohepatic circulation of bile acids is interrupted, the negative feedback of the conversion of cholesterol to bile acids is eliminated, more bile acids are excreted in the feces and the enzyme 7α-hydroxylase is activated. Thereby, more cholesterol is converted to bile acids in an effort to maintain its pool. These changes, reduce the concentration of cholesterol in the liver cells. Thus, the synthesis of LDL receptors is increased and then more LDL-C is cleared by the liver. Consequently, the plasma levels of cholesterol are reduced (Mayes, 1988 and Linscheer and Vergroesen, 1988).

Some substances, such as cholestyramine resin and some dietary fibers, which can enhance the elimination of bile acids in the feces, appear to be of great significance on the control of hypercholesterolemia. These factors are discussed in the following sections (1.3 and 1.4.3).

1.2. CHOLESTEROL RELATED DISEASE

The excess of cholesterol in the body can lead to cholesterol deposition in plaques within the intimal lining of the arteries resulting in atherosclerosis and thereby enhancing the risk for coronary heart disease (CHD), the leading cause of death in the Western World (Rifai, 1986; Fletcher and Rogers, 1985 and Consensus Conference, 1985).

Hypercholesterolemia in the majority of the cases results from elevation of the plasma low-density lipoprotein cholesterol (LDL-C) which appears to be the most atherogenic lipoprotein (Grundy et al., 1986 and Grundy, 1987). The LDL-C when in excess in the blood is deposited in the arterial wall leading to atherosclerotic plaque (Consensus Conference, 1985). In fact several studies have shown that an elevated level of LDL-C but a reduced level of high-density lipoprotein-cholesterol (HDL-C) is related to an increased risk of CHD (Rifai, 1986; Ginter et al., 1982b; Anderson and Tietyen-Clark, 1986 and Grundy, 1986).

Epidemiological studies have supported a causal relationship between blood cholesterol levels and CHD (Consensus Conference, 1985; Rifkind, 1986 and Canadian Consensus Conference on Cholesterol, 1988). Prospective studies such as the Framingham Heart Study, the Pooling Project, and the Multiple Risk Factor Intervention Trial (MRFIT) have described the link between elevated blood

cholesterol levels and CHD. The higher the blood cholesterol, the greater the risk (Vega and Grundy, 1987 and Anderson and Tietyen-Clark, 1986). According to the National Institutes of Health Cholesterol Consensus Conference (1985) a moderate hypercholesterolemia is considered when plasma cholesterol levels exceeds 240 mg/dl. Approximately 15% of middle-age American men appear to present plasma cholesterol levels exceeding this level (Vega and Grundy, 1987).

The Lipid Research Clinics Coronary Primary Trial, CPPT (1984) demonstrated that the reduction of blood cholesterol could significantly decrease the incidence of myocardial infarction. It was shown that each 1% reduction in plasma cholesterol is associated with approximately a 2% reduction in CHD rates. Based on the evidence from all these epidemiologic and interventional studies it is apparent that elevated blood cholesterol levels, especially cholesterol from LDL are closely related to the incidence of CHD.

1.3. DRUGS CONTROLLING BLOOD CHOLESTEROL

A variety of hypocholesterolemic drugs have been used to lower plasma cholesterol levels. These include the bile acid sequestrants (cholestyramine and colestipol), probucol, nicotinic acid, clofibrate and gemfibrozil (Consensus Conference, 1985). In addition, mevinolin is

another class of drugs currently available for treating hypercholesterolemic patients (Miettinen and Kasàniemi, 1986 and McMurray, 1983). Almost all these agents are known to present side effects (Miettinen and Kasaniemi, 1986; Grundy, 1986 and Consensus Conference, 1985).

Bile acid sequestrants have been largely studied in clinical trials and in experiments. They bind bile acids in the intestine thus preventing their reabsorption (Miettinen and Kasàniemi, 1986 and Ginter et al., 1981). However, they may produce or worsen constipation as well as hemorrhoids, and interfere with normal fat absorption, consequently reducing the absorption of fat soluble vitamins, such as A,D,E, and K (Canadian Pharmaceutical Association, 1987). Probucol lowers plasma total cholesterol by 10 to 25% and the LDL-cholesterol levels by 10 to 15%, probably due to enhanced catabolism of LDL (Einarsson and Angelin, 1986). However, this agent also decreases the HDL-cholesterol, which is considered to be a protective factor for CHD, and may raise the serum triglycerides (Kasaniemi and Grundy, 1984; Miettinen and Kasaniemi, 1986 and Canadian Pharmaceutical Association, 1987). The use of nicotinic acid (niacin) in large doses (2-4 g/day) in the treatment of hypercholesterolemia, can precipitate many unpleasant side effects such as flushing of the face and skin reactions (Miettinen and Kasàniemi, 1986).

Fibric acid derivatives, such as clofibrate and gemfibrozil, most widely used hypolipidemic agents, have

been reported to reduce plasma concentrations of cholesterol by reducing the activity of HMG-CoA reductase (McMurray, 1983). They can also decrease the VLDL-C levels by lipolysis (Einarsson and Angelin, 1986 and Mayes, 1988). Two large long term trials have shown that the users of clofibrate had increased frequency of gallstone disease, since it increases the cholesterol saturation in bile (Einarsson and Angelin, 1986 and Canadian Pharmaceutical Association, 1987). Compactin and mevinolin are other drugs, which lowers serum cholesterol levels through inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis (Illingworth and Sexton, 1984; McMurray, 1983 and Grundy, 1986). Since, mevinolin has been used only recently, its potential toxic effects have not yet been reported (Grundy, 1986).

Since most hypocholesterolemic agents are potentially toxic if they are used on a long-term basis, dietary modifications should be the choice of health care teams, who are responsible for the treatment of the hypercholesterolemic patients.

1.4. NUTRITIONAL FACTORS AFFECTING CHOLESTEROL METABOLISM

Most mild forms of hypercholesteromia appear to be related to diet (Grundy, 1984). Therefore, it is of interest to examine the role of dietary factors on

cholesterol metabolism.

1.4.1. CHOLESTEROL, SATURATED FATTY ACIDS AND TOTAL CALORIC INTAKE

The major dietary factors that appear to be responsible for the high levels of cholesterol among Western populations are saturated fatty acids and cholesterol (Grundy, 1986 and Grundy, 1987). The work of Goldstein and Brown (1984) on the LDL receptors was fundamental to clarify the mechanism by which dietary fat influences plasma cholesterol levels (Grundy, 1986). This mechanism is summarized in Fig. 1.3.

Saturated fatty acids and dietary cholesterol reduce the LDL receptor activity, consequently the fractional catabolic rate (FCR) of LDL-C is decreased (Rifkind, 1986; Vega and Grundy, 1987 and Goldstein and Brown, 1984). This mechanism is explained by a down regulation of LDL receptor activity, a feedback control. The synthesis of LDL receptors is controlled by amounts of cholesterol in the liver cells, so when the concentration of cholesterol inside those cells increases, the LDL receptors will be suppressed (Goldstein and Brown, 1984, Grundy, 1987 and Pyorala, 1987) . The uptake of LDL from plasma into the cell is reduced thus increasing the plasma LDL concentrations (Rifkind, 1986 and Grundy, 1986). The VLDL remnants enter the liver in a slower rate, being converted

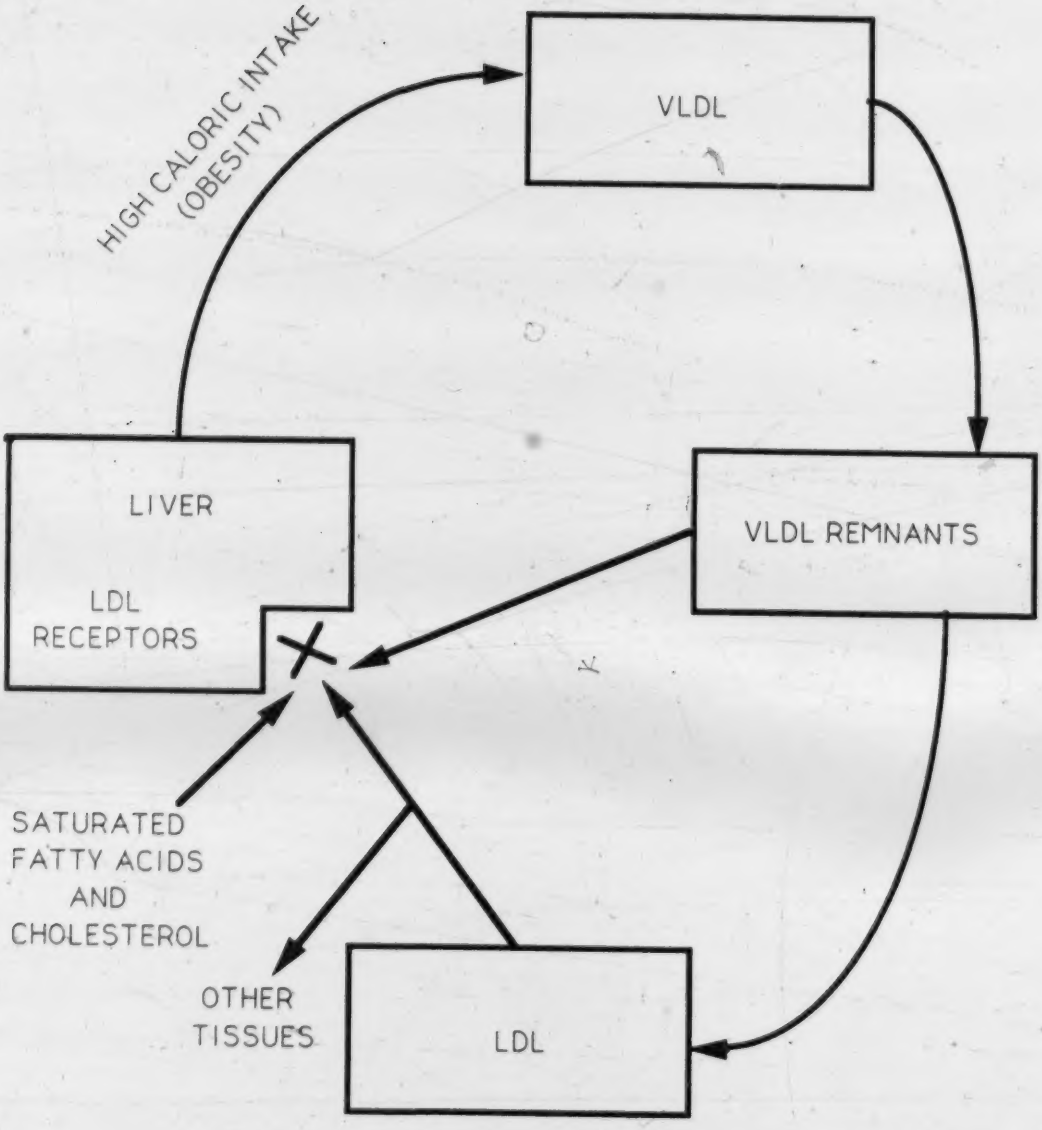

Figure 1.3 Effect of a diet high in saturated fats, cholesterol and calories (obesity) on the metabolism of lipoproteins (modified after Grundy, 1986).

to LDL in elevated amounts and also increasing the plasma LDL levels (Goldstein and Brown, 1984 and Grundy, 1986).

Total caloric intake is another factor that can affect plasma levels of cholesterol. A high caloric intake (obesity) leads to an overproduction of VLDL, consequently raising the LDL levels, as shown in Fig. 1.3 (Grundy, 1987).

1.4.2. POLYUNSATURATED FATTY ACIDS

Polyunsaturated fatty acids such as linoleic acid (omega-6 series), have been reported to lower plasma cholesterol levels. Their mechanism of action appears to be related to the parallel reduction of LDL-C levels and particles number (Grundy, 1987). However, the long-term effects of polyunsaturated fatty acids as a substitute for saturated ones is unknown. It has been reported that they can lower HDL-C, and potentiate carcinogenesis as well as immunosuppression in laboratory animals (Grundy, 1986).

Polyunsaturated fatty acids with long-chain fatty acids of the omega-3 series, particularly eicosapentaenoic acid (EPA) present in some fish oils, have been reported to lower VLDL and LDL in normal subjects (Bierman and Chait, 1988). The association of EPA with low incidence of atherosclerosis has been observed in populations on fish-based diets (Fletcher and Rogers, 1985 and Bierman and Chait, 1988). Studies have shown that high dietary levels

of EPA result in prolonged bleeding time and decreased platelet aggregation (Fletcher and Rogers, 1985). However the role of the omega-3 series of polyunsaturated fatty acids on the treatment and prevention of hyperlipidemia is not completely clarified (Bierman and Chait, 1988).

1.4.3. DIETARY FIBER

Dietary fibers, especially water-soluble ones such as pectin (Kay and Truswell, 1977; Lee and Han, 1985; Anderson and Tietyen-Clark, 1986; Sable-Amplis et al., 1987 and Kritchevsky, 1987), oat meal (Shinnick et al., 1988) and guar-gum (Superko et al., 1988) have been reported to lower serum cholesterol in humans and in experimental animals. However, wheat bran, an insoluble fiber, appears to have no effect on plasma and liver cholesterol levels (Kritchevsky, 1987).

Pectin (polygalacturonic acid compounds) is a mixture of complex carbohydrates (Baig and Cerda, 1981) that is present in vegetables and fruits (Wolthuis et al., 1980). This non-absorbable polymer appears to be capable of forming gel in the intestine with water and acid (Hillman et al., 1986; Ginter et al., 1982b and Southgate et al., 1986). The precise mechanism by which pectin exerts its hypocholesterolemic effect has not yet been clarified (Kay, 1986 and Kritchevsky, 1987). The major hypothesis is that pectin binds bile acids in the intestine, thus decreasing

their enterohepatic recirculation (Anderson and Tietyen-Clark, 1986 and Kay and Truswell, 1977). More bile acids will be excreted in the stool consequently less bile acids return to the liver. This will prevent the negative feedback of bile acid synthesis when in presence of elevated bile acid pool, so more cholesterol will be catalyzed to bile acids (Kay and Truswell, 1977; Ginter et al., 1982b; Lee and Han, 1985; Ahrens et al., 1986 and Anderson and Tietyen-Clark, 1986). It has been also suggested that pectin may interfere directly with cholesterol absorption via intestinal morphofunctional changes (Judd and Truswell, 1985 and Vahouny et al., 1988).

Because pectin reduces the total plasma and liver cholesterol concentrations, it may also alter the lipid composition of lipoproteins. Evidence to date indicates that the influence of pectin on the lipoprotein profile in plasma is variable. Thus, high-density lipoprotein cholesterol (HDL-C) concentration in plasma has been found to be unaltered in normocholesterolemic humans and animals (Judd and Truswell, 1982 and Schneeman et al., 1984), while hypercholesterolemic rats appear to be associated with an increased HDL-C concentration (Chen and Anderson, 1979 and Chen et al., 1981), when feeding a diet containing 10% pectin. Unlike in rats, feeding a high pectin diet to hypercholesterolemic minipigs (Ahrens et al., 1986) was found to lower plasma levels of both HDL-C and low-density lipoprotein cholesterol (LDL-C). Another study (Vigne et

al., 1987) in rats fed either a low-fat diet or a high-fat cholesterol diet, both supplemented with pectin, reported that LDL-C and very low-density lipoprotein cholesterol (VLDL-C) in plasma were significantly reduced only when a low fat diet was given; however the HDL-C was not changed. It seems that the conflicting results among these studies might be due to a number of variables such as experimental diets, initial cholesterol status, duration of the study, and species used.

1.4.4. VITAMIN C

The previous review of cholesterol synthesis, transport and catabolism was intended to provide the basis for a better understanding of the mechanism by which vitamin C (L-ascorbic acid) intervenes into cholesterol metabolism.

In the last two decades the influence of vitamin C on cholesterol metabolism has been the subject of a number of investigations (Ginter, 1975; Ginter and Bobek, 1981; Fidanza et al., 1982; Holloway and Rivers, 1984 ; Erden et al., 1985 and O'Brien and McMurray, 1988). First studies of the relationship among L-ascorbic acid, lipid metabolism and atherosclerosis were carried out either on animals synthesizing vitamin C (rats and rabbits) or on vitamin C depleted guinea pigs, with scurvy symptoms (Ginter et al., 1982b). These conditions do not reflect the situation in

human nutrition. It is very difficult to interpret any metabolic disorder encountered in scorbutic animals since it is a complex state. The animals may present loss of weight, anorexia, hemorrhage and a complex metabolic unbalance (Basu and Schorah, 1982 and Ginter et al., 1982a). A more suitable model for biochemical research was developed by Ginter (1975). A vitamin C-free diet was given to guinea pigs during two weeks in order to deplete their tissue stores. Then a maintenance dose of ascorbic acid (0.5 mg of vitamin C daily) was given during 30 weeks which prevented symptoms of scurvy, thus low vitamin C tissue levels were maintained leading to a chronic latent hypovitaminosis C (Ginter, 1975 and Ginter et al., 1982b).

Extensive studies, using guinea pigs with subclinical vitamin C deficiency (Ginter, 1975 and Ginter and Bobek, 1981) and inherently scorbutic rats (Horio et al., 1987 and Kono et al., 1988), have been carried out demonstrating elevated plasma and liver cholesterol concentrations, a decreased HDL-C level, an increased plasma cholesterol half-life, an increased ratio of total cholesterol/high density lipoprotein (HDL-cholesterol), and increased cholesterol concentrations in gallbladder bile.

Several studies (Ginter, 1975; Bjorkhem and Kallner, 1976 and Holloway and Rivers, 1984) have shown that ascorbic acid is necessary for cholesterol transformation to bile acid. It has emerged from these studies that ascorbic acid stimulates the activity of the enzyme

7α-hydroxylase, via the microsomal cytochrome P450, which mediates the transformation of cholesterol into 7α-hydroxycholesterol (Fig. 1.4). It has been observed that marginal vitamin C depletion in guinea pigs (Basu and Schorah, 1982) and in inherently scorbutic rats (Horio et al., 1989) leads to reduced activity of 7α-hydroxylase. The exact mechanism by which ascorbic acid intervenes into the turnover of microsomal cytochrome P450 is not yet clarified (Ginter et al., 1982b).

The influence of ascorbic acid on the metabolism of the lipoproteins has also been investigated. It has been reported that the increase of total cholesterol in guinea pigs with marginal vitamin C deficiency is related to an increase of LDL-C, since the concentration of HDL-C is only slightly reduced (Ginter et al., 1982b). A recent experiment was carried out (Ginter and Jurcovicova, 1987) to elucidate the effect of marginal vitamin C deficiency on plasma LDL turnover of guinea pig. LDL receptors were measured in "vivo" by determining the rate of disappearance of ^{125}I-labelled LDL from the circulation. The fractional catabolic rate (FCR) for LDL was also calculated. It was found that vitamin C deficient guinea pigs had higher levels of liver and plasma cholesterol, and longer half-life as well as lower FCR of plasma LDL compared to those of the control animals. It is claimed (Ginter and Jurcovicova, 1987) that a chronic state of vitamin C deficiency may lead to decreased vitamin C in hepatocytes,

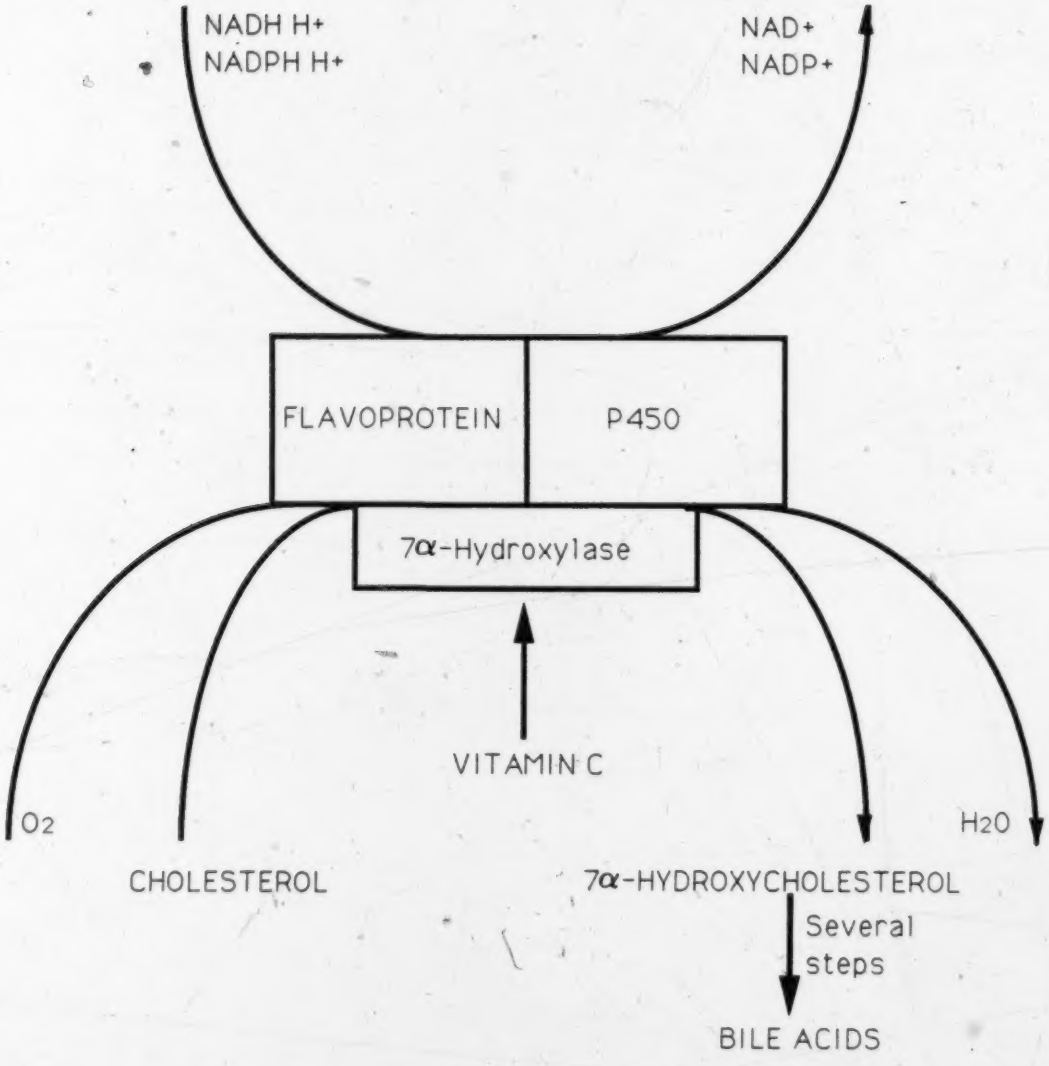

Figure 1.4 Microsomal hydroxylating enzyme system and cytochome P450 (modified after Basu and Schorah, 1982).

reduced activity of cholesterol 7α-hydroxylase in the liver, reduced transformation of cholesterol into bile acids, accumulation of cholesterol in the liver, decreased production of LDL receptors on the liver cell surface and increased plasma LDL levels. Data on the relationship between ascorbic acid and cholesterol metabolism in humans are not clear (Ginter et al., 1982b and Jacques et al., 1987), however it is believed that hypovitaminosis C may cause similar biochemical and pathological changes in humans (Basu and Schorah, 1982). The most striking hypocholesterolemic effects of vitamin C have been observed in hypercholesterolemic elderly people (Ginter et al., 1982a).

Supplementation of vitamin C (500-4000 mg/day) given to normal and hypercholesterolemic subjects has been found to reduce plasma total and LDL-C levels as well as increase HDL-C levels (Ginter, 1975; Ginter, 1979; Horsey et al., 1981; Fidanza et al., 1982; Ginter et al., 1982a and Ginter et al., 1982b). In contrast, other intervention trials have reported no changes on plasma total and lipoproteins cholesterol when vitamin C has been supplemented (Peterson et al., 1975 and Johnson and Obenshain, 1981).

Observational studies, mostly carried out in healthy elderly subjects, have examined the relationship between cholesterol and plasma vitamin C. They have reported a positive association between plasma vitamin C and HDL-C levels (Bates et al., 1977; Burr et al., 1982 and Jacques

et al., 1987) as well as an inverse correlation between plasma vitamin C and total cholesterol levels (Burr et al., 1982 and Grego and LaRocca, 1982). However, other studies have found that plasma vitamin C levels were not correlated with either HDL-C (Hooper et al., 1983) or total cholesterol levels (Hooper et al., 1983 and Jacques et al., 1987). The controversial findings among these studies might be due to methodological shortcomings, since some of them have small number of subjects and no controls. Another possible explanation for these findings is that the vitamin C status was not similar for all populations studied. It has been observed that the hypocholesterolemic effect of vitamin C depends on the initial plasma levels of this vitamin (Ginter et al., 1982a,b), thus the greater the plasma cholesterol levels the more effective seems to be the hypocholesterolemic effect of vitamin C.

The precise role of the vitamin C on cholesterol metabolism is not yet clarified (O'Brien and McMurray, 1988). However, the observed link between them appears to be of potential clinical importance, especially for the target populations, such as elderly and hypercholesterolemic patients.

1.5. CONCLUSION AND OBJECTIVES OF THE PRESENT STUDY

There appears to be a substantial amount of evidence suggesting that both ascorbic acid and soluble dietary fiber components lower cholesterol levels. Essentially, ascorbic acid lowers cholesterol by stimulating cholesterol 7α-hydroxylase, the rate limiting enzyme for cholesterol degradation. The action of soluble dietary components (pectin in particular), on the other hand, is mediated through binding and eliminating bile acids, the breakdown products of cholesterol.

Most of the long-term interventional studies have shown that the administration of ascorbic acid (300-1000 mg/day) to hypercholesterolemic persons reduces the plasma cholesterol level; however, after approximately 6 months it tends to return to its pre-high levels (Ginter et al., 1981). The ascorbic acid-induced bile acid synthesis may lead to an increased body pool size of bile acids (Harris et al., 1979). Increased quantities of these organic acids may, however, return to the liver via the enterohepatic recirculation resulting in a decreased activity of 7α-hydroxylation of cholesterol (as a feed-back inhibition). It is, therefore, important that this feed-back effect is eliminated if hypercholesterolemia is to be effectively lowered. One possibility is the simultaneous administration of soluble dietary fiber components, which binds bile acids in the gut. The proposed

mechanism (Ginter et al., 1982b) of a synergistic effect of ascorbic acid and pectin in lowering cholesterol levels is shown on Fig. 1.5. A synergistic effect of ascorbic acid and cholestyramine (a bile acid binding agent) in lowering blood cholesterol has been reported (Ginter et al., 1981). Only one study (Ginter et al., 1979) has as yet been reported testing the combined action of ascorbic acid and pectin. In this study, the pectin associated lowering effect on liver and plasma cholesterol levels was found to be enhanced with ascorbic acid in guinea pigs. It is not possible to suggest, however, if the effect is synergistic or simply additive. They also tested this dietary combination in hypercholesterolemic humans (Ginter et al., 1982a). Eleven hyperlipemic outpatients receiving a daily preparation of 15 g of citrus pectin and 450 mg of ascorbic acid had a marked decrease of almost 20% in their plasma cholesterol levels after 6 weeks of treatment. These preliminary results clearly justify further exploration.

The present study was designed to investigate the short and long-term effects of dietary pectin and vitamin C on the lipid status of mice receiving a high cholesterol diet. This study was carried out in animals fed their diets either isocalorically or ad libitum in order to determine whether there is a direct influence of the amount of food intake on the lipid status.

The animals used in this study were mice, which have the ability to synthesize ascorbic acid in the body. Hence,

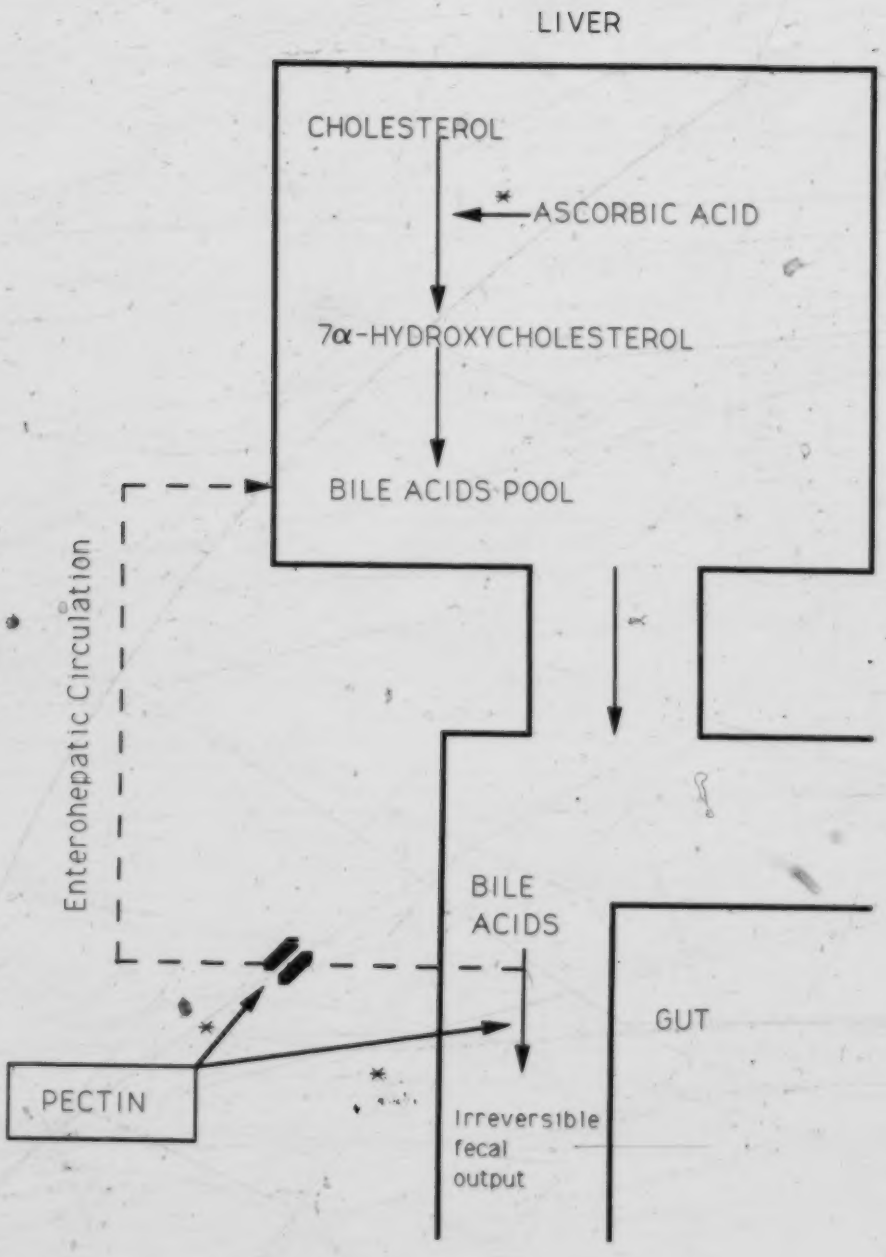

Figure 1.5 Proposed mechanism of a synergistic effect between ascorbic acid and pectin in lowering cholesterol levels (modified after Ginter et al., 1982b)

it may be argued that ascorbic acid administration to these animals may result in suppression of its synthesis due to feed back inhibition. It is therefore, possible that the animals treated with ascorbic acid may not be truly exposed to any larger amount of this vitamin than the untreated control animals. However, it is thought that the amount of ascorbic acid administered is large enough to exceed the amount that mice may synthesize in the body.

The hypocholesterolemic effects of soluble dietary fiber components such as pectin, guar-gum and oat gum have been documented in experimental animals and humans (Kritchevsky, 1987; Shinnick et al., 1988 and Superko et al., 1988). This effect appears to be most significant when the initial cholesterol status is elevated (Hillman et al., 1985 and Vahouny and Cassidy, 1986). Ascorbic acid administration has also been reported to significantly decrease plasma cholesterol levels in subjects with hypercholesterolemia, but not in normocholesterolemic subjects (Ginter et al., 1982a). Hence, in this study the mice were fed a 1% cholesterol diet to induce hypercholesterolemia.

The proposed study was undertaken essentially to investigate:

1. What influence does either pectin or ascorbic acid make on the total cholesterol and lipoprotein status?

2. If there is a synergistic effect between ascorbic acid and pectin in lowering cholesterol status.

3. If the cholesterol lowering effect of ascorbic acid and pectin is influenced by dietary intake and/or absorption of dietary cholesterol.

CHAPTER 2. METHODOLOGY

2.1. ANIMALS AND DIETS

Male adult ICR albino mice (University of Alberta), weighing 25-30 g, were used throughout. The animals were individually housed in plastic cages. The room was temperature controlled ($25^{o}C \pm 2$) and it was on a 12-hour light-dark cycle. All mice were fed a pellet diet (Wayne rat chow, Allied Mills Inc.) for a week before being fed an experimental semi-synthetic diet (Table 2.1).

2.1.1. STUDY 1: SHORT-TERM EFFECTS

The animals were randomly divided into five groups (Table 2.2) of six each. Group 1 was fed a diet containing no added cholesterol (diet A) and used as normocholesterolemic controls. Group 2 and 3 were fed a diet containing 1% cholesterol without (diet B) or with 5% pectin (diet C); group 2 was used as hypercholesterolemic controls. Group 4 was fed the diet B plus dietary ascorbic acid, AA (TEROCHEM) every day in their drinking water (1 mg/ml). Group 5 was fed the diet C plus AA in drinking water, as described for group 4. All animals had free access to fresh water (average intake of 8-10 ml/day) and their respective diets for 4 weeks; body weights were recorded once a week. At the end of the period, the animals were fasted for 12

TABLE 2.1 DIET COMPOSITION (%)

Ingredients	Diet A	Diet B	Diet C
Casein[1]	20	20	20
Olive oil[2]	10	10	10
Cholesterol[3]	0	1	1
Vitamin premix[4]	2	2	2
Mineral mixture[5]	3.5	3.5	3.5
Cellulose powder[6]	1.5	1.5	0
Pectin powder[7]	0	0	5
Cornstarch[8]	63	62	58.5

[1]Casein from TEKLAD Test Diets. [2]Olive oil (No.102502). from ICN Biochemicals Divisions. [3]Cholesterol (No.101380) from ICN Biochemicals Divisions. [4]Vitamin Mix A.O.A.C. (No.40055), from TEKLAD Test Diets, composition (g/Kg of diet when added at 2%): vitamin A and D powder, 0.08; dry vitamin E acetate, 0.4; menadione (vitamin k_3), 0.010; choline dihydrogen citrate (41% choline), 9.7561; p-aminobenzoic acid, 0.2; inositol, 0.2; D-calcium pantothenate, 0.08; riboflavin, 0.016; thiamin- HCl, 0.01; pyridoxine HCl, 0.01; folic acid, 0.004; biotin, 0.0008; vitamin B_{12} (0.1% trituration in mannitol), 0.06=0.00006g of vitamin B_{12}; dextrose, anhydrous, 9.0931. [5]Mineral Mix, Bernhart-Tomarelli (No.170750), from TEKLAD Test Diets, composition (g/Kg): $CaCO_3$, 21; $CaHPO_4$, 735.0; MgO, 25; K_2HPO_4, 81; K_2SO_4, 68.0; NaCl, 30.6; Na_2HPO_4, 21.4; $Cu_2C_6H_4O_7.2\ 1/2H_2O$, 0.46; ferric citrate (16.7% Fe), 5.58; manganese citrate (13.9% Mn), 8.35; KI, 0.0072; zinc citrate, 1.33; citric acid, 2.2728. [6]Cellulose from TEKLAD Test Diets. [7]Pectin Citrus, (No.102587), from ICN Biochemicals Divisions, composition: methoxy content NLT, 6.1%; galacturonic acid NLT, 74%; LOD, NMT, 10%. [8]Cornstarch from ICN Biochemicals Divisions.

Table 2.2 EXPERIMENTAL GROUPS AND THEIR RESPECTIVE DIETS

GROUPS	DIETS
1	Diet A; normal cholesterol
2	Diet B; 1% cholesterol
3	Diet C; 1% cholesterol + 5% pectin
4	Diet B + ascorbic acid in drinking water (1mg/ml)
5	Diet C + ascorbic acid in drinking water (1mg/ml)

hours, anesthetized with chloroform and then blood was collected through cardiac puncture in heparinized tubes. Plasma was separated by centrifugation (Beckman-J2-21 centrifuge, 3000 rpm for 10 min at -4 ^{o}C). The livers were quickly removed, excised, weighed and homogenized with 1.15% KCl in a ratio of 1:4 (w/v). The separated plasma and homogenized liver samples were frozen (-40^{o}C) until analyses.

2.1.2. STUDY 2: LONG-TERM EFFECTS

The animals were divided into five groups with six animals each. Group 1 and 2 were fed the normocholesterolemic (diet A) and hypercholesterolemic (diet B) diets, respectively, as in study 1. Group 3 was fed a diet containing 5% pectin (Table 2.1: diet C). Group 4 and 5 were fed the diets B and C (Table 2.1) respectively, plus 1 mg AA/ml drinking water as described in study 1. All groups were fed their respective diets for 12 weeks. Fresh water and food were supplied ad libitum daily, and body weight was recorded once a week. After 12 weeks the animals were sacrificed; blood and liver samples were collected as described in study 1.

2.1.3. STUDY 3: PAIR-FED CONTROL STUDY

The mice were divided into three groups with six

animals each. Group 1 received a hypercholesterolemic diet (Table 2.1: diet B) and group 2 received a diet containing 5% pectin (Table 2.1: diet C). Group 3 received the diet B (Table 2.1), and water given ad libitum containing 1 mg AA/ml as described in study 1. All animals were pair fed. After 4 weeks of pair feeding, the animals were placed in metabolic cages. Twenty-four hour feces were collected, weighed and frozen (-40°C) until analyzed. The dried fecal samples were used to determine their cholesterol content. After the collection of fecal samples, the animals were returned to their plastic cages. All animals continued to be fed isocalorically. On week 8 the animals were killed by the same procedure described in studies 1 and 2. Plasma was collected and stored at -40°C until analyzed.

2.2. ANALYTICAL METHODS

In order to investigate the effects of pectin and ascorbic acid on the lipid status in mice fed a high cholesterol (1%) diet, plasma total cholesterol (T-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), liver total cholesterol and fecal cholesterol were determined. Hepatic levels of ascorbic acid were also determined to investigate the storage condition of this vitamin on the groups of animals with and without AA supplementation.

2.2.1. DETERMINATION OF PLASMA TOTAL CHOLESTEROL

Using Boehringer Mannheim kit, plasma total cholesterol (cat# 237574), was assayed enzymatically as described by Kattermann et al. (1984). Enzymatic cholesterol assay methods were developed in the beginning of last decade (Allain et al., 1974). They have replaced the previous laborious and time consuming chemical methods for simpler, quicker and precise procedures (Kattermann et al., 1984).

The test principle reactions are as follows:

$$\text{cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{cholesterol} + \text{RCOOH} \qquad [2.1]$$

$$\text{cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol}} \Delta^4\text{-cholestenone} + H_2O_2 \qquad [2.2]$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-mono-imino) - phenazone} + 4H_2O_2 \qquad [2.3]$$

The 4-(p-benzoquinone-mono-imino) - phenazone, is a color complex. The amount of color produced is directly proportional to the total cholesterol content of the sample.

A solution containing cholesterol esterase, cholesterol

peroxidase and cholesterol oxidase was used as cholesterol reagent (solution A). An aliquot of plasma (0.02 ml) and 2.00 ml of reagent solution were pipetted into a test tube. Another tube containing 2.00 ml of reagent solution (solution A) was used as reagent blank. The tubes were mixed on a vortex mixer and incubated for 10 minutes at 20-25 ^{o}C. Using a PERKIN ELMER UV/VIS Spectrophotometer Lambda 3 B, the absorbance of the samples was measured against the reagent blank, at 500 nm. All samples were measured in duplicate.

The concentration, C (mmol/l), of cholesterol in the samples was calculated as following:

$$C = 14.9 \times \text{Absorbance of the sample} \qquad [2.4]$$

For control of accuracy, Precinorm-L control sera (Boehringer Mannheim, lot No. 159882) was used in all biochemical parameters measured in plasma.

2.2.2. DETERMINATION OF PLASMA HDL-CHOLESTEROL

HDL-C was assayed enzymatically (Boehringer Mannheim kit) based on the method described by Lopes-Virella et al. (1977), in which HDL-C is measured as the cholesterol in the clear supernatant solution after the precipitation of apo β-containing lipoproteins. The precipitation occurs by the addition of a polyanion (phosphotungstic acid) and a

divalent cation (magnesium ions) to the plasma sample.

Aliquots of plasma (0.2 ml) and 0.5 ml of HDL-C precipitant (Boehringer Mannheim, cat# 543004) were pipetted into 1.5 ml micro centrifuge tubes. The tubes were mixed on a vortex mixer, allowed to stand for 10 minutes at room temperature and centrifuged (refrigerated microcentrifuge, Microcentaur) for 2 minutes at 12000 rpm. The clear supernatant was separated within two hours.

Aliquots of the clear supernatant (0.2 ml) and 2.0 ml of cholesterol reagent solution were pipetted into test tubes. Two milliliters of reagent solution (solution A) and 0.2 ml of redistilled water were pipetted into another test tube, and used as reagent blank. The tubes were mixed on a vortex mixer and incubated for 10 minutes at 20-25 ^{o}C. The absorbance of the samples was read against the reagent blank at 500 nm on a PERKIN-ELMER UV/VIS Lambda 3 B Spectrophotometer, within one hour. All samples were measured in duplicate.

The HDL-C concentration, C (mmol/l), was calculated as follows:

$$C = 219.2 \times \text{Absorbance of the sample} \qquad [2.5]$$

2.2.3. DETERMINATION OF LDL CHOLESTEROL

The cholesterol content of LDL was determined indirectly using the method described by Friedwald et al.

(1972). This method requires the measurement of plasma total cholesterol, triglycerides and HDL-C. The following formula was used to estimate LDL-C (mmol/l):

$$LDL\text{-}C = T\text{-}C - (TG/2.2) - HDL\text{-}C \qquad [2.6]$$

This formula is based on the assumption that VLDL-C approximates plasma true triglycerides divided by 2.2, however, it cannot be applicable to plasma samples with chylomicrons, type III hyperlipoproteinemia and containing triglycerides concentration exceeding 400 mg/100 ml. Those restrictions appeared not to be present on the plasma samples measured in this experiment, thus the obtained values can be considered reliable.

2.2.4. DETERMINATION OF TRIGLYCERIDES

Triglyceride concentrations in plasma samples were measured by an enzymatic colorimetric method (Boehringer Mannheim kit, cat# 701 912) (Wahlefeld, 1974). This method is based on the enzymatic hydrolysis of triglycerides with subsequent determination of the liberated glycerol as follows:

$$\text{triglycerides} + 3H_2O \xrightarrow{\text{lipase}} \text{glycerol} + 3RCOOH \qquad [2.7]$$

$$\text{glycerol} + \text{ATP} \xrightarrow[\text{kinase}]{\text{glycerol}} \text{glycerol-3-phosphate} + \text{ADP} \quad [2.8]$$

$$\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{glycerol phosphate oxidase}} \text{dihydroxyacetone phosphate} + H_2O_2 \quad [2.9]$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-mono-imino)-phenazone} + 2H_2O + HCl \quad [2.10]$$

An aliquot (0.02 ml) of plasma and 2.00 ml of triglyceride reagent solution (solution B) were pipetted into a test tube. Two milliliters of reagent solution (solution B) are pipetted into another test tube as reagent blank. The test tubes were mixed on a vortex mixer and incubated at 20-25 ^{o}C for 10 minutes. The absorbance of the samples was read against reagent blank at 500 nm on a PERKIN-ELMER UV/VIS Lambda 3 B Spectrophotometer, within one hour. All samples were measured in duplicate.

The concentration, C (mmol/l), of triglycerides in the samples was calculated as follows:

$$C = 8.66 \times \text{Absorbance of the sample} \quad [2.11]$$

The free glycerol was corrected by substracting 0.11 mmol/l

from the calculated value above.

2.2.5. DETERMINATION OF THE LIVER TOTAL CHOLESTEROL

The method of Watson (1960) was used for the determination of liver cholesterol. This is a colorimetric method which avoids extraction and hydrolysis steps. This procedure is based on a modification of the method described by Pearson et al. (1953), in which 2,5-dimethylbenzene sulphonic acid replaces p-toluene sulphonic acid. All reagents employed were of analytical grade. Pure cholesterol (Sigma) dissolved in glacial acetic acid was used as the standard (range: 2.59-10.35 µmol/ml). The absorbance of standards increased in direct proportion to their concentrations (Fig. 2.1).

Aliquots of 0.1 ml of liver homogenate were placed into test tubes; 0.1 ml of distilled water and standard (10.35 µmol/ml) were also set up for the blank and standard respectively. Glacial acetic acid (0.1 ml) was added to the sample and the blank, but 0.1 ml of distilled water was added to the standard instead. Two and a half milliliters of reagent mixture (3 volumes of acetic acid anhydride with 1 volume of the 2,5-dimethylbenzene sulphonic acid solution and 1 volume of glacial acetic acid) was added into each tube, and mixed using a vortex mixer. Each tube was allowed to cool for 10-15 minutes. Concentrated sulphuric acid (0.3 ml) was added to each tube, and the contents were agitated

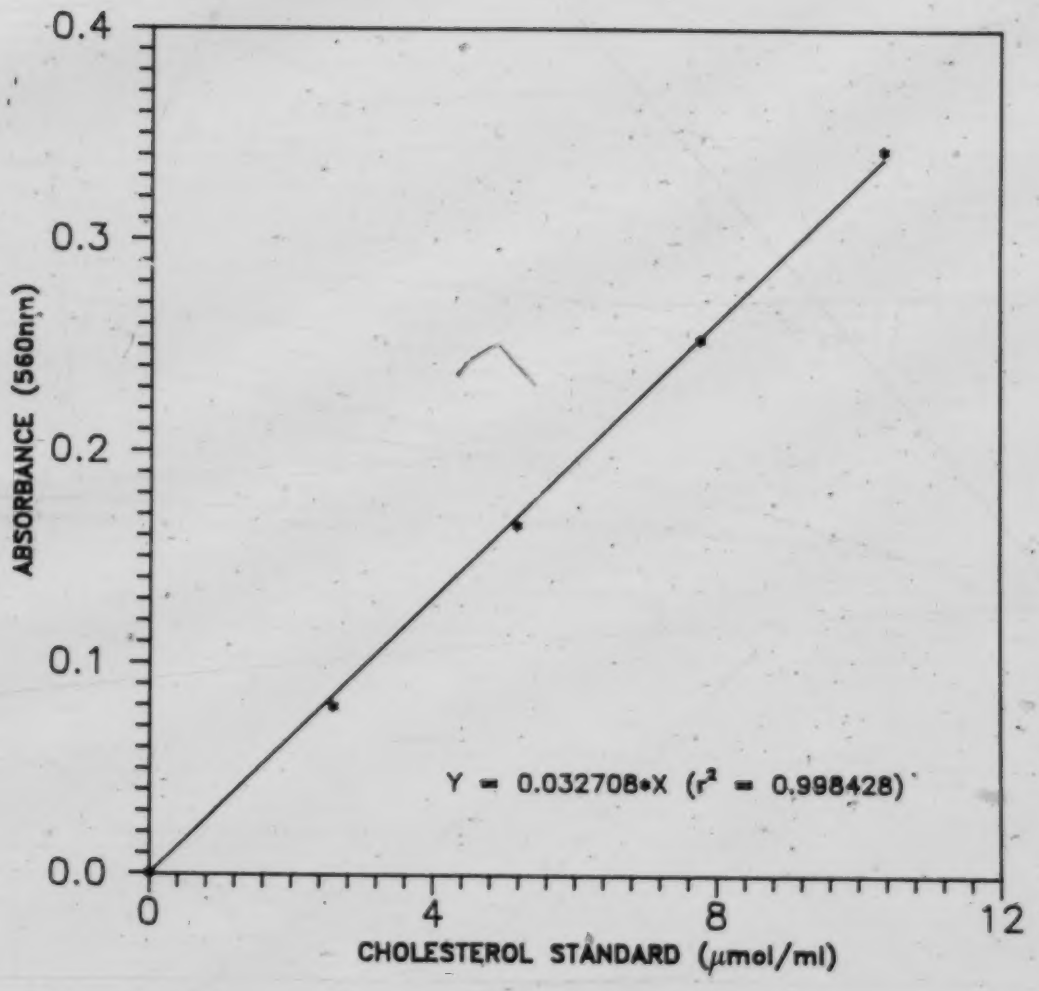

Figure 2.1 Standard curve for cholesterol determination

immediately until all precipitates had dissolved. The test tubes were allowed to stand in the dark for color development. Using a PERKIN-ELMER UV/VIS Lambda 3 B Spectrophotometer, the absorbance of each sample was measured at 560 nm. All samples were measured against the blank 20 minutes (±2 min) after the addition of sulphuric acid. At least one standard was included for each run to check for the variation and the stability of the machine. All samples were measured in duplicate and the average reading was used in the calculation of the amount of cholesterol in the liver.

2.2.6. DETERMINATION OF FECAL CHOLESTEROL

Fecal samples were dried overnight, weighed and homogenized with KCl (1.15%) in a 1:40 (w/v) dilution. Pure cholesterol (Sigma) dissolved in glacial acetic acid at range of 2.59 to 10.35 µmol/ml was used as the standard for fecal cholesterol determination. Fecal homogenates were extracted according to Folch et al. (1957). The intensity of absorbance increased linearly with the concentration of the standards (Fig 2.1). An aliquot (0.5 ml) of fecal homogenate was mixed with 9.0 ml of chloroform-methanol (2:1, v/v) mixture on a vortex mixer; and then mixed thoroughly with 2.5 ml of distilled water. The mixture was allowed to separate into two phases by standing overnight. The obtained

upper phase and the fluffy layers were discarded. An aliquot of 1.0 ml was taken from the lower layer which contains cholesterol. The solvent was removed by passing a stream of dry N_2. Cholesterol was then determined according to the method of Watson (1960) as described on section 2.2.5.

2.2.7. DETERMINATION OF HEPATIC CONCENTRATION OF ASCORBIC ACID (VITAMIN C)

Hepatic concentrations of total ascorbic acid were assayed by the method of Roe end Keuther (1943) as modified by Bessey (1950). In this method, ascorbic acid is coupled with 2-4-dinitrophenylhydrazine (2:4DNPH) to form a 2-4-dinitrophenylhydrazone, which is converted into a red compound and determined colorimetrically. Liver samples were homogenized with KCl (1.15%) in a 1:4 dilution. Pure ascorbic acid (Sigma) dissolved in distilled water at range of 0.011 to 0.114 µmol/ml, was used as the standard for ascorbic acid analysis. As shown in Fig. 2.2, the intensity of the absorbance increased linearly with the concentration of these standards.

2.3. STATISTICAL ANALYSIS

Means and standard error of the means (SEM) were determined for all groups of animals in studies 1, 2, and 3. Data were analyzed by using one-way analysis of variance

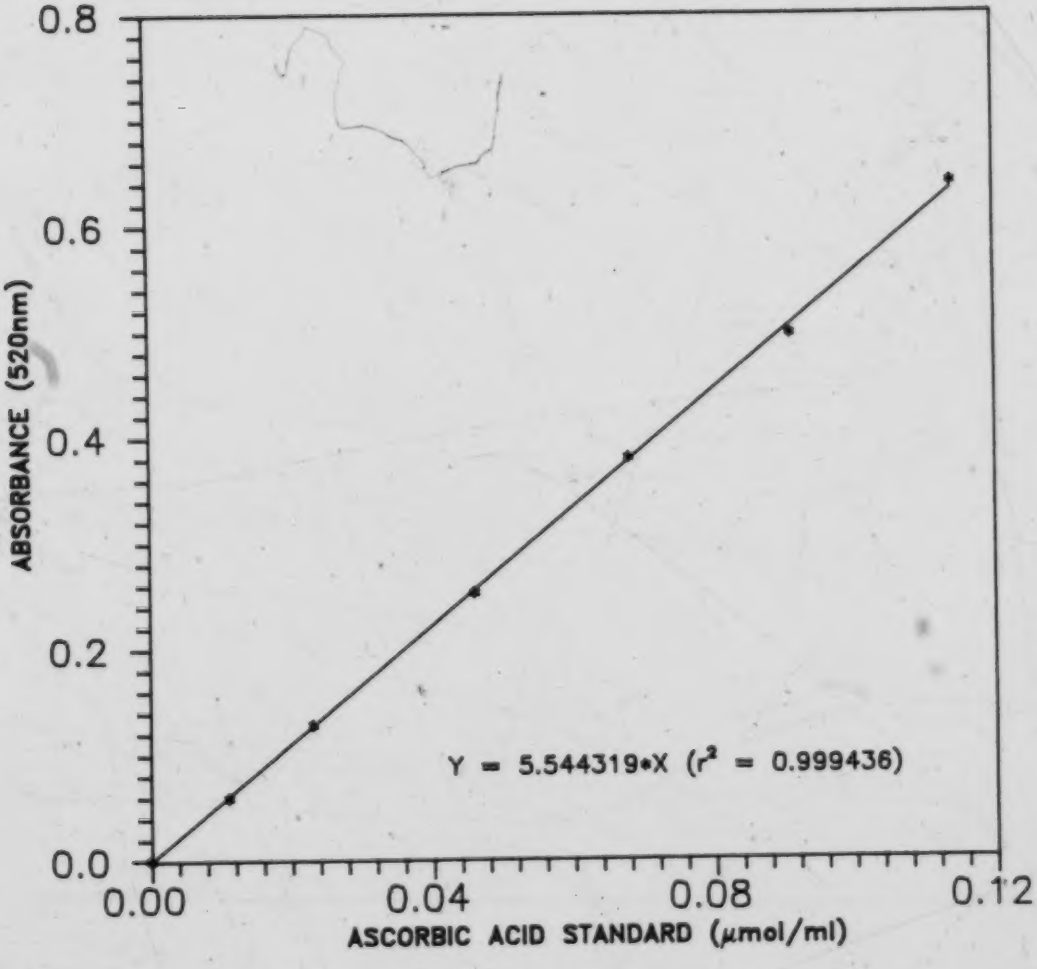

Figure 2.2 Standard curve for ascorbic acid determination

(ANOVA). When significant differences were detected, Fisher LSD, multiple-comparison method (Winer, 1971) was used to determine which mean values were significantly different. In this present study, the level of significance (α) considered was 0.05.

2.4. HISTOLOGICAL ANALYSIS

For histological analysis, liver samples from all five groups of animals fed their respective diets (Table 2.2) were quickly removed, weighed, sliced and placed on a 10% formaline solution. Those samples were sent to the Pathology Department (University of Alberta Hospital) where further histological preparation and analyses were carried out. Liver sections were taken and stained with Harris's haematoxylin and eosin. Photomicrographs, using Zeiss Photomicroscope III, of the livers sections were obtained.

CHAPTER 3. EXPERIMENTAL RESULTS

This study investigated the effects of pectin and ascorbic acid either alone or in combination, on the lipid status in male mice receiving a semi-synthetic diet containing 1% cholesterol. The animals were fed various dietary regimes for 4 and 12 weeks. The results of this study were compared with those of animals receiving either no added cholesterol (control A) or 1% cholesterol (control B). These are discussed in 4 sections.

3.1. EFFECT OF HIGH CHOLESTEROL DIET (1%) ON THE LIPID STATUS IN MICE

Feeding a 1% cholesterol diet (control B) for either 4 or 12 weeks did not appear to have any significant effect on body weight, compared to that of the animals receiving no added cholesterol, control A (Table 3.1). The liver weight relative to body weight, however, was found to be increased significantly ($P < 0.05$) in animals fed a high cholesterol diet for 4 weeks. The difference in liver weight between groups A and B, however, disappeared at 12 weeks (Table 3.1). The cholesterol-associated increase in liver weight at 4 weeks was also evident by the fact that the liver of the cholesterol treated animals looked larger in size and was paler in color compared to the livers of the controls (Plate 3.1 A and B). Furthermore, animals fed a high cholesterol

TABLE 3.1 Body weight gain, and the liver weight relative to body weight in mice fed a high cholesterol (H-C) diet for 4 and 12 weeks.

Groups	Body weight gain(g) weeks		Liver wt/Body wt x 100 weeks	
	4	12	4	12
Normal: control A	3.2 ± 0.59^{a}	3.8 ± 0.93^{a}	4.9 ± 0.30^{a}	5.0 ± 0.20^{a}
H-C: control B	3.6 ± 0.94^{a}	4.3 ± 1.05^{a}	5.8 ± 0.32^{b}	4.9 ± 0.21^{a}

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

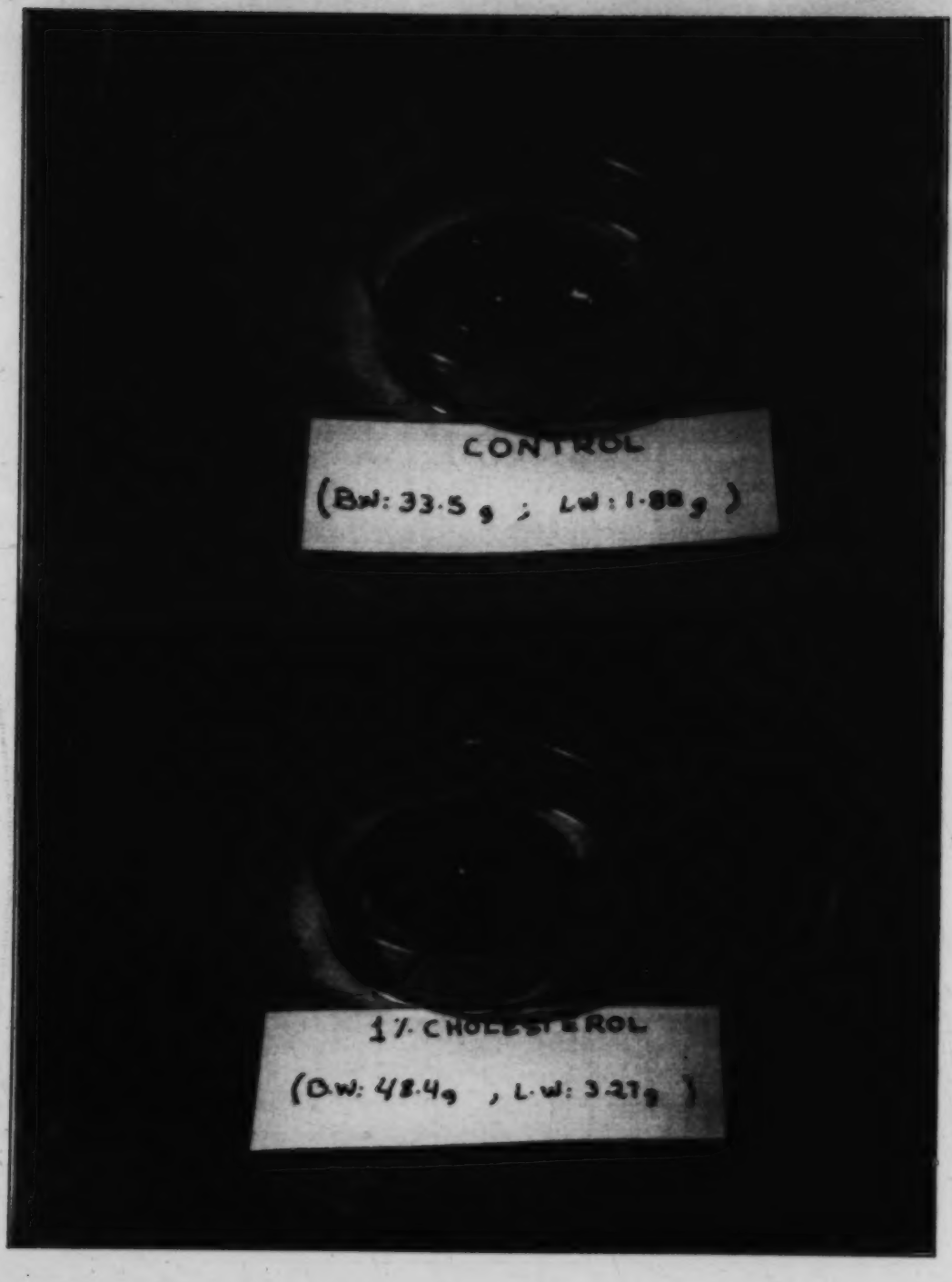

Plate 3.1 Photographs of the livers from animals; (A) fed a normal diet (control A); (B) fed a 1% cholesterol diet (control B)

diet (control B) for 4 weeks were accompanied by increased hepatic concentrations of T-C (Table 3.3). The plasma concentrations of T-C and lipoproteins were also found to be markedly elevated in these animals (Table 3.2).

Although feeding a high cholesterol diet for 12 weeks did not have any effect on the liver weight (Table 3.1), the plasma T-C and lipoproteins (Table 3.2) as well as the hepatic levels of total cholesterol (Table 3.3) of these animals were found to be markedly elevated at 4 weeks. The biochemical evidence of the hypercholesterolemic liver in animals fed a high cholesterol diet for 12 weeks was also confirmed by the histologic section of the liver, showing increased lipid accumulation compared to those on the normal diets, control A (Plate 3.2 A and B).

3.2. EFFECT OF PECTIN (5%) ON THE LIPID STATUS IN HIGH CHOLESTEROL (1%) FED MICE

Mice fed a 1% cholesterol diet without (control B) and with pectin for either 4 or 12 weeks were not statistically different in body weights, compared to those of the control animals, receiving a semi-synthetic diet with no added cholesterol and pectin, control A (Table 3.4). The addition of pectin (5%) to the diet of the 1% cholesterol-fed mice, resulted in reductions in the liver weight relative to body weight, but not significantly, compared to those of the control diet (Table 3.4).

TABLE 3.2 Plasma lipid profiles in mice fed a high cholesterol (1%) diet for 4 and 12 Weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
			4 weeks		
Normal: control A	3.48[a] ±0.22	2.16[a] ±0.23	2.29[a] ±0.11	0.36[a] ±0.08	5.13[a] ±0.62
H-C: control B	5.60[b] ±0.36	2.48[a] ±0.50	3.13[b] ±0.31	1.35[b] ±0.10	2.43[b] ±0.34
			12 weeks		
Normal: control A	3.51[a] ±0.32	1.74[a] ±0.20	2.36[a] ±0.20	0.35[a] ±0.04	6.80[a] ±0.30
H-C: control B	4.65[b] ±0.32	1.49[a] ±0.25	3.36[b] ±0.20	0.63[b] ±0.06	5.50[a] ±0.34

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.3 Hepatic cholesterol levels in mice fed a high cholesterol (1%) diet for 4 and 12 weeks.

Groups	µmol/g liver	µmol/liver
	4 weeks	
Normal (control A)	21.7 ± 1.3[a]	37.20 ± 3.5[a]
H-C (control B)	62.2 ± 7.4[b]	138.35 ± 28.1[b]
	12 weeks	
Normal (control A)	21.2 ± 0.5[a]	35.70 ± 2.2[a]
H-C (control B)	84.9 ± 6.4[b]	151.80 ± 14.4[b]

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

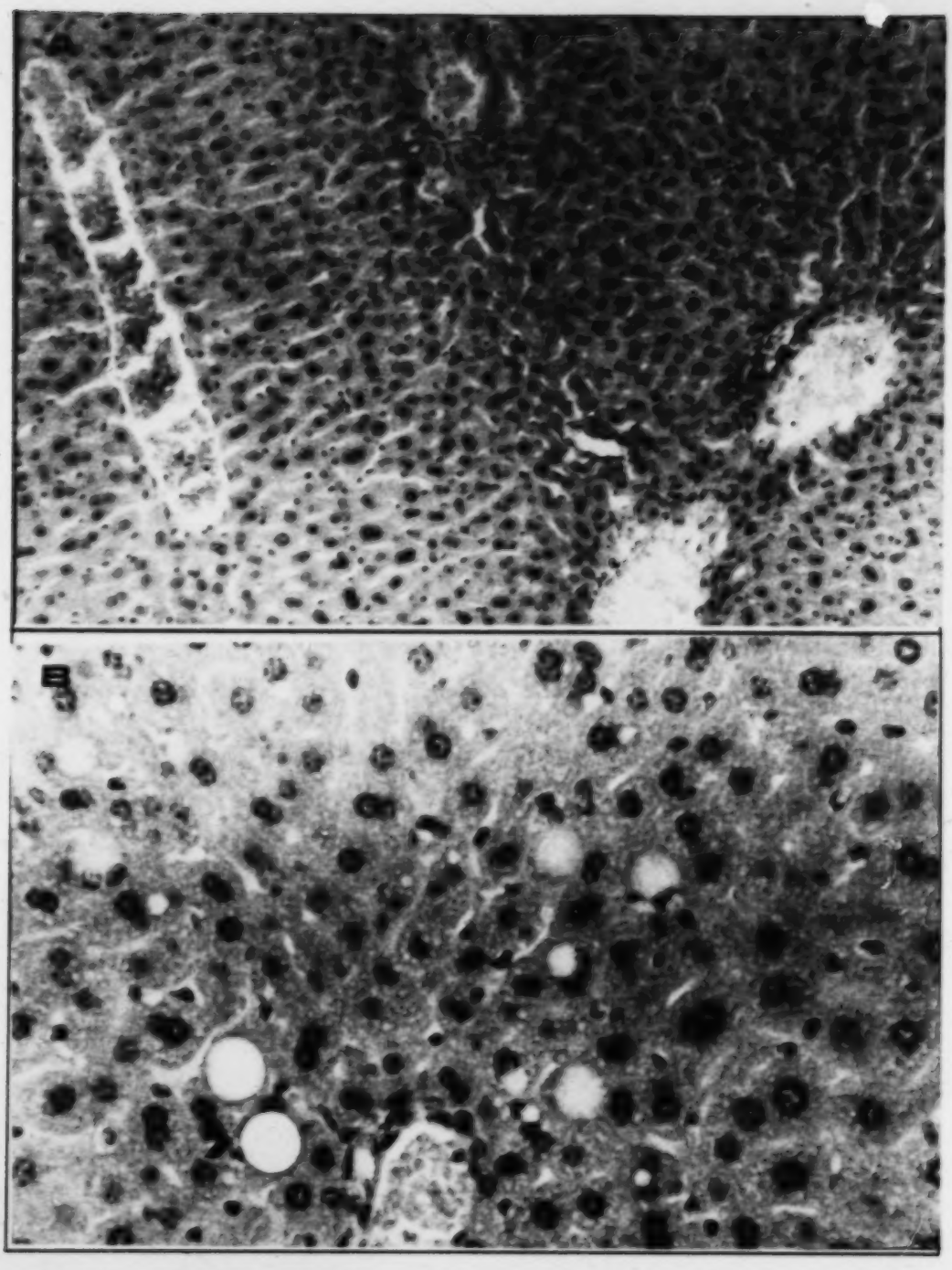

Plate 3.2 Photomicrographs of the livers from animals; (A) fed a normal diet (control A) for 12 weeks (magnification 10X); (B) fed a 1% cholesterol diet (control B) for 12 weeks (magnification 25X).

TABLE 3.4 Effect of pectin (Pe) on the body weight gain and liver weight relative to body weight in high cholesterol (1%) fed mice for 4 and 12 weeks.

Groups	Body weight gain(g) weeks		Liver wt/Body wt x 100 weeks	
	4	12	4	12
Normal: control A	3.2^{a} ±0.59	3.8^{a} ±0.93	4.9^{a} ±0.30	5.0^{a} ±0.20
H-C: control B	3.6^{a} ±0.94	4.3^{a} ±1.05	5.8^{b} ±0.32	4.9^{a} ±0.21
H-C+Pe	3.5^{a} ±1.06	3.5^{a} ±0.85	5.2^{ab} ±0.21	4.6^{a} ±0.18

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

Table 3.5 displays the plasma lipid profiles of the animals after 4 weeks on their respective experimental diets (study 1). The 1% cholesterol-fed mice receiving pectin (5%) had significantly ($P < 0.05$) lower levels of plasma T-C, TG, and LDL-C than those mice receiving a 1% cholesterol diet but without pectin (control B). These results were in parallel with the hepatic levels of T-C (Table 3.6); this was true in terms of its concentration (μmol/g liver) as well as its absolute amount (μmol/liver). It was of interest that feeding a high cholesterol diet (control B) resulted in increased level of plasma HDL-C, compared with animals receiving a normocholesterolemic diet (control A). The administration of pectin (5%) for 4 weeks resulted in a decrease in the high-cholesterol-associated rise in plasma HDL-C levels as well as an increase in HDL-C/LDL-C ratio (Table 3.5). The decrease in HDL-C concentration and the increase in its ratio to LDL-C were not, however, statistically significant.

The plasma lipid profiles of the animals after 12 weeks (study 2) on their experimental diets are shown in Table 3.7. The animals receiving a diet containing 1% cholesterol with pectin, had their levels of plasma T-C and LDL-C reduced in relation to those of the control B. Unlike study 1, the addition of pectin (5%) to the 1% cholesterol diet did not affect the plasma concentrations of TG. On the other hand, feeding the pectin diet for 12 weeks resulted in significantly ($P < 0.05$) decreased levels of plasma HDL-C as

TABLE 3.5 Plasma lipid profiles in mice fed a high cholesterol (1%) diet with (5%) and without pectin for 4 weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
Normal: control A	3.48^{a} ±0.22	2.16^{ab} ±0.23	2.29^{a} ±0.11	0.36^{a} ±0.08	5.13^{a} ±0.62
H-C: control B	5.60^{b} ±0.36	2.48^{a} ±0.50	3.13^{b} ±0.31	1.35^{b} ±0.10	2.43^{b} ±0.34
H-C+Pe	4.08^{a} ±0.60	1.35^{b} ±0.12	2.63^{ab} ±0.19	0.85^{c} ±0.06	3.16^{ab} ±0.23

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.6 Hepatic cholesterol levels in mice fed a high cholesterol (1%) diet with (5%) and without pectin for 4 weeks.

Groups	µmol/g liver	µmol/liver
Normal (control A)	21.7 ± 1.3^{a}	37.2 ± 3.5^{a}
H-C (control B)	62.2 ± 7.4^{b}	138.4 ± 28.1^{b}
H-C+Pe	39.1 ± 1.5^{c}	74.4 ± 6.9^{a}

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.7 Plasma lipid profiles in mice fed a high cholesterol (1%) diet with (5%) and without pectin for 12 weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
Normal: control A	3.51^{a} ±0.32	1.74^{a} ±0.20	2.36^{a} ±0.20	0.35^{a} ±0.04	6.80^{a} ±0.30
H-C: control B	4.65^{b} ±0.32	1.49^{a} ±0.25	3.36^{b} ±0.20	0.63^{b} ±0.06	5.50^{a} ±0.34
H-C+Pe	2.87^{a} ±0.20	1.33^{a} ±0.11	2.07^{a} ±0.14	0.19^{a} ±0.04	12.21^{b} ±1.69

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

well as increased HDL-C/LDL-C ratios (Table 3.7). Hepatic cholesterol levels, as in study 1 (Table 3.6), were reduced when pectin was added to the 1% cholesterol diet (Table 3.8). Histological examination of the livers taken from the 1% cholesterol-fed mice (control B) for 12 weeks revealed an increase in number of fat vacuoles (Plate 3.2B). Plate 3.3 shows clearly that the increase in hepatic fat vacuoles did not occur when pectin was added at 5% level in a 1% cholesterol diet. It is noteworthy that the histologic section of the liver of the animals receiving 1% cholesterol diet with pectin (Plate 3.3) appeared to be similar to those receiving a normocholesteremic diet, control A (Plate 3.2A).

Since all animals were fed their respective diets ad libitum, it is possible that the pectin associated changes in hepatic and plasma lipid status were the reflection of total food intake. In order to determine whether the effects were truly due to the pectin intake, animals of different groups in subsequent studies were fed their diets isocalorically. Table 3.9 shows that the pair-fed mice (study 3), receiving a 1% cholesterol diet supplemented with 5% pectin for 8 weeks, had their plasma T-C, TG, and HDL-C levels similar to those of the animals which had the access to the diets ad libitum. Thus, the plasma indices were all reduced in the pectin-fed animals when compared to those receiving a 1% cholesterol diet without pectin (control B). The fecal weights (wet and dry), as well as fecal cholesterol concentrations were found to be similar between

TABLE 3.8 Hepatic cholesterol levels in mice fed a high cholesterol diet (1%) with (5%) and without pectin for 12 weeks.

Groups	µmol/g liver	µmol/liver
Normal (control A)	21.2 ± 0.5^{a}	35.7 ± 2.2^{a}
H-C (control B)	84.9 ± 6.4^{b}	151.8 ± 14.4^{b}
H-C+Pe	42.9 ± 4.7^{c}	63.2 ± 8.2^{a}

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

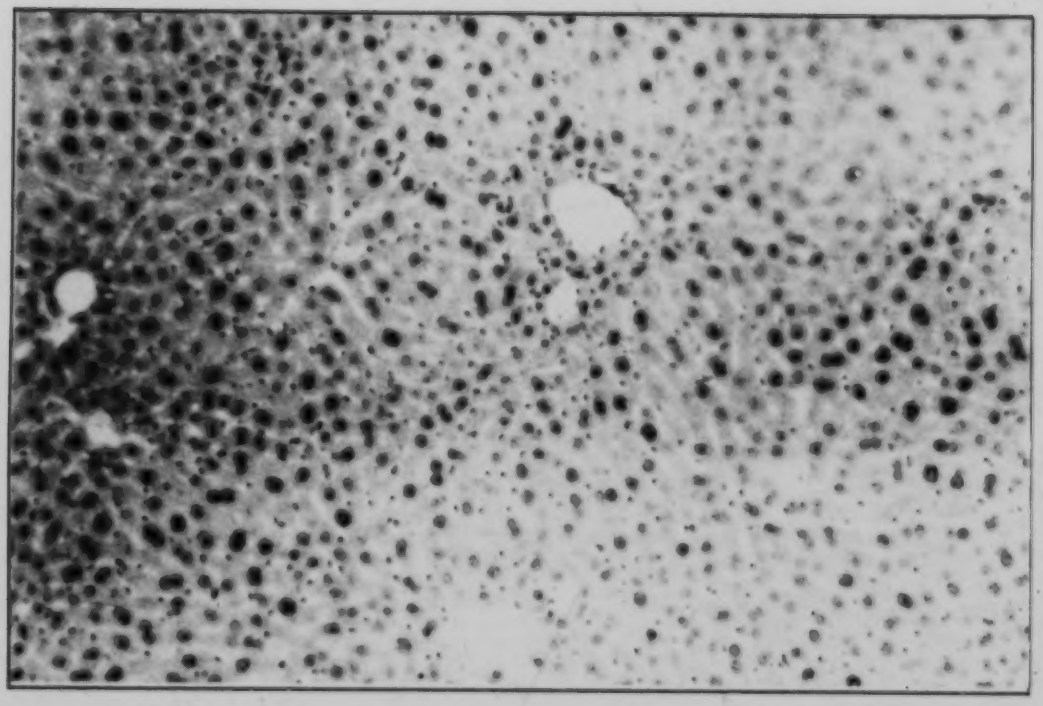

Plate 3.3 Photomicrograph of the liver from an animal fed a 1% cholesterol diet with pectin (5%) for 12 weeks (magnification 10X).

TABLE 3.9 Plasma lipid profiles in mice fed an isocaloric high cholesterol (1%) diet with (5%) and without pectin for 8 weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
H-C: control B	3.96^{a} ±0.32	1.04^{a} ±0.01	2.66^{a} ±0.30	0.83^{a} ±0.02	3.18^{a} ±0.28
H-C+Pe	2.74^{b} ±0.36	0.73^{b} ±0.06	1.87^{b} ±0.15	0.54^{a} ±0.33	5.03^{a} ±1.31

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

the pair-fed groups receiving 1% cholesterol diet either alone (control B) or with pectin for 4 weeks (Table 3.10).

3.3. EFFECT OF ASCORBIC ACID ON THE LIPID STATUS IN HIGH CHOLESTEROL FED MICE

Mice fed diets containing 1% cholesterol without (control B) and with ascorbic acid (supplemented in their drinking water, 1 mg/ml) for either 4 or 12 weeks, had similar body weights compared to those of the control animals receiving a normocholesterolemic diet without ascorbic acid supplementation, control A (Table 3.11). The cholesterol-associated rise in liver weight relative to body weight observed in animals fed a high cholesterol diet for 4 weeks, remained unchanged when ascorbic acid (1 mg/ml) was administered to these animals (Table 3.11). Feeding animals the high cholesterol diet and ascorbic acid for 12 weeks, however, resulted in an increase in the liver weight relative to body weight compared to that of either control A or control B (Table 3.11).

Table 3.12 shows the plasma lipid profiles in animals fed a high cholesterol diet plus ascorbic acid for 4 and 12 weeks. At 4 weeks, the plasma levels of T-C, TG and LDL-C were significantly ($P < 0.05$) less than those of the control B. Although not significanty, the ascorbic acid supplementation also decreased HDL-C concentration. These reductions in ascorbic acid treated animals were accompanied

TABLE 3.10 Fecal weight and cholesterol concentration in mice fed an isocaloric high cholesterol diet with (5%) and without pectin for 4 weeks.

Groups	Fecal weight (g)		Fecal cholesterol (µmol/day)
	wet	dry	
H-C (control B)	0.19[a] ±0.03	0.17[a] ±0.03	49.2[a] ±8.7
H-C+Pe	0.21[a] ±0.03	0.18[a] ±0.02	41.0[a] ±5.3

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.11 Effect of ascorbic acid (AA) on the body and liver weights in high cholesterol (1%) fed mice for 4 and 12 weeks.

Groups	Body weight gain(g) weeks		Liver wt/Body wt x 100 weeks	
	4	12	4	12
Normal: control A	3.2^{a} ±0.59	3.8^{a} ±0.93	4.9^{a} ±0.30	5.0^{a} ±0.20
H-C: control B	3.6^{a} ±0.94	4.3^{a} ±1.05	5.8^{b} ±0.32	4.9^{a} ±0.21
H-C+AA	3.8^{a} ±0.75	4.1^{a} ±0.96	5.8^{b} ±0.21	5.4^{b} ±0.19

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.12 Effect of ascorbic acid on plasma lipid profiles in high cholesterol (1%) fed mice for 4 and 12 weeks.

Groups	T-C	TG	HDL-C	LDL-C	HDL-C / LDL-C
	(mmol/l)				
	4 weeks				
Normal: control A	3.48^{a} ±0.22	2.16^{ab} ±0.23	2.29^{a} ±0.11	0.36^{a} ±0.08	5.13^{a} ±0.62
H-C: control B	5.60^{b} ±0.36	2.48^{a} ±0.50	3.13^{b} ±0.31	1.35^{b} ±0.10	2.43^{b} ±0.34
H-C+AA	4.27^{c} ±0.19	1.65^{b} ±0.16	2.95^{b} ±0.08	0.58^{a} ±0.14	5.46^{a} ±0.46
	12 weeks				
Normal: control A	3.51^{a} ±0.32	1.74^{a} ±0.20	2.36^{a} ±0.20	0.35^{a} ±0.04	6.80^{a} ±0.30
H-C: control B	4.65^{b} ±0.32	1.49^{ab} ±0.25	3.36^{b} ±0.20	0.63^{b} ±0.06	5.50^{a} ±0.34
H-C+AA	3.32^{a} ±0.30	0.95^{b} ±0.10	2.29^{a} ±0.20	0.60^{b} ±0.08	4.00^{a} ±0.55

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

by an increase in the HDL-C/LDL-C ratio. Animals receiving a high cholesterol diet plus ascorbic acid for 12 weeks, also had lower ($P < 0.05$) plasma levels of T-C and HDL-C as well as TG (but not significantly) than those of the animals fed a high cholesterol diet alone (control B). However, high cholesterol-fed animals when treated with ascorbic acid for 12 weeks, had similar plasma levels of LDL-C as well as HDL-C/LDL-C ratios compared to those of the control B.

The daily administration of ascorbic acid to high cholesterol-fed animals for 4 weeks did not reduce either the concentration or the absolute amount of cholesterol in the liver, compared to those animals receiving a high cholesterol diet alone, control B, (Table 3.13). The high cholesterol-fed animals treated with ascorbic acid for 12 weeks, had even higher ($P < 0.05$) hepatic cholesterol concentrations than those of the control B (Table 3.13). The histological analysis of the livers was in parallel with the biochemical findings, thus it indicated that animals receiving a high cholesterol diet plus ascorbic acid for 12 weeks, had greater lipid accumulation in their livers (Plate 3.4) than those of the animals receiving a high cholesterol diet alone, control B (Plate 3.2B).

Table 3.14 shows the plasma lipid profiles in mice receiving isocalorically a high cholesterol diet with and without ascorbic acid supplementation for 8 weeks. Pair-fed animals treated with ascorbic acid, had similar plasma levels of T-C, HDL-C and LDL-C to those receiving a high

TABLE 3.13 Effect of ascorbic acid on the hepatic cholesterol concentration in high cholesterol (1%) fed mice for 4 and 12 weeks.

Groups	µmol/g liver	µmol/liver
	4 weeks	
Normal (control A)	21.7 ± 1.3[a]	37.2 ± 3.5[a]
H-C (control B)	62.2 ± 7.4[b]	138.4 ± 28.1[b]
H-C+AA	66.1 ± 6.8[b]	144.0 ± 21.2[b]
	12 weeks	
Normal (control A)	21.2 ± 0.5[a]	35.7 ± 2.2[a]
H-C (control B)	84.9 ± 6.4[b]	151.8 ± 14.4[b]
H-C+AA	108.7 ± 4.8[c]	202.7 ± 16.1[c]

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

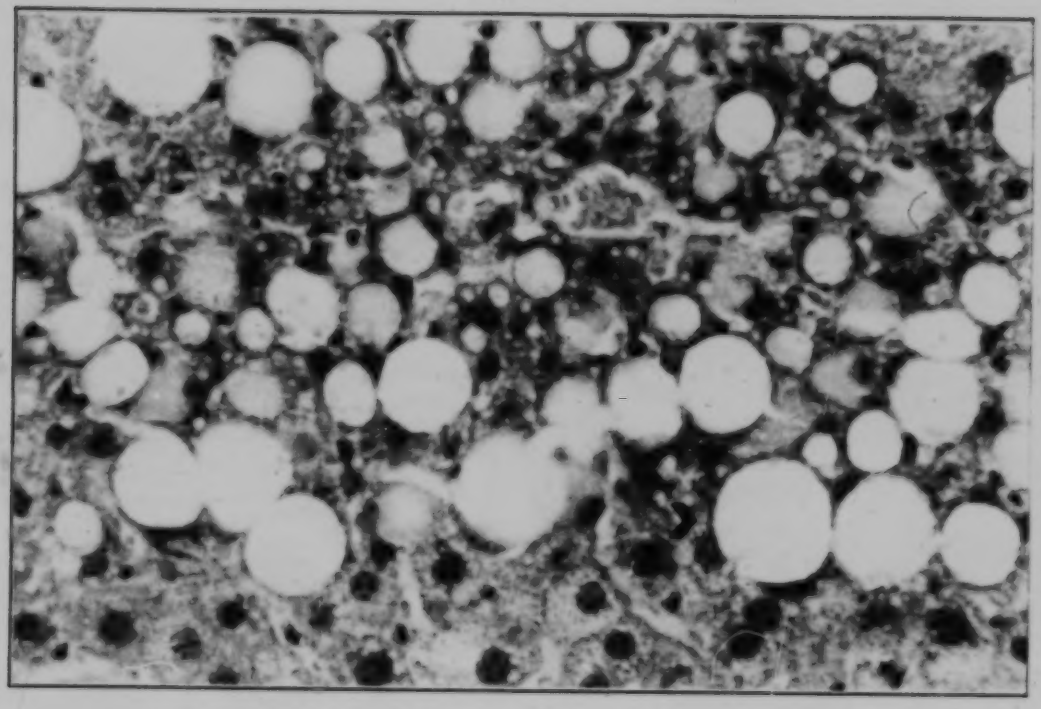

Plate 3.4 Photomicrograph of the liver from an animal fed a 1% cholesterol diet with ascorbic acid supplemented in drinking water (1 mg/ml) for 12 weeks (magnification 25X).

TABLE 3.14 Plasma lipid profiles in mice fed an isocaloric high cholesterol (1%) diet either alone or with ascorbic acid for 8 weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
H-C: control B	3.96^{a} ±0.32	1.04^{a} ±0.01	2.66^{a} ±0.30	0.83^{a} ±0.02	3.18^{a} ±0.28
H-C+AA	4.71^{a} ±0.40	0.81^{b} ±0.08	3.16^{a} ±0.94	1.18^{a} ±0.33	4.77^{a} ±1.75

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

cholesterol diet alone. The plasma TG concentration, however, was significantly decreased ($P < 0.05$), whereas the HDL-C/LDL-C ratio increased, but not significantly, in animals supplemented with ascorbic acid. The fecal weights, as well as fecal cholesterol were similar in both groups (Table 3.15).

3.4. EFFECT OF PECTIN IN COMBINATION WITH ASCORBIC ACID ON THE LIPID STATUS IN HIGH CHOLESTEROL FED MICE

Administration of ascorbic acid alone for 12 weeks resulted in a significantly increased liver weight relative to body weight when compared with that of the either control B or pectin-fed group (Table 3.16). The ascorbic acid-associated increase in liver weight was reversed but not significantly when ascorbic acid was administered in combination with pectin.

The animals treated with either ascorbic acid or pectin for 4 weeks were significantly associated ($P < 0.05$), with decreased levels of T-C, TG and LDL-C when compared with those of the control B diet (Table 3.17). In addition ascorbic acid but not pectin, resulted in an increase in HDL-C/LDL-C ratio in these animals. However, simultaneous administration of ascorbic acid and pectin, failed to demonstrate any synergistic effects in terms of their hypolipidemic actions when given individually. At 12 weeks the animal's response to the treatment was somewhat similar

TABLE 3.15 Fecal weight and cholesterol concentration in mice fed an isocaloric high cholesterol diet either alone or with ascorbic acid for 4 weeks.

Groups	Fecal weight (g)		Fecal cholesterol (µmol/day)
	wet	dry	
H-C (control B)	0.19[a] ±0.03	0.17[a] ±0.03	49.2[a] ±8.7
H-C+AA	0.19[a] ±0.03	0.17[a] ±0.03	51.9[a] ±8.1

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.16 Effect of pectin and ascorbic acid either alone or in combination on the body and liver weights in high cholesterol (1%) fed mice for 4 and 12 weeks.

Groups	Body weight gain(g) weeks		Liver wt/Body wt x 100 weeks	
	4	12	4	12
H-C (control B)	3.6^{a} ±0.94	4.3^{a} ±1.05	5.8^{a} ±0.32	4.9^{a} ±0.21
H-C+Pe	3.5^{a} ±1.06	3.5^{a} ±0.85	5.2^{a} ±0.21	4.6^{a} ±0.18
H-C+AA	3.8^{a} ±0.75	4.1^{a} ±0.96	5.8^{a} ±0.21	5.4^{b} ±0.19
H-C+Pe+AA	3.6^{a} ±0.62	4.3^{a} ±1.32	5.4^{a} ±0.13	5.0^{ab} ±0.32

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.17 Effect of pectin and ascorbic acid either alone or in combination on plasma lipid profiles in mice fed a high cholesterol (1%) diet for 4 weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
H-C (control B)	5.60^{a} ±0.36	2.48^{a} ±0.50	3.13^{a} ±0.31	1.35^{a} ±0.10	2.43^{a} ±0.34
H-C+Pe	4.08^{b} ±0.60	1.35^{b} ±0.12	2.63^{a} ±0.19	0.85^{b} ±0.06	3.16^{a} ±0.23
H-C+AA	4.27^{b} ±0.19	1.65^{b} ±0.16	2.95^{a} ±0.08	0.58^{b} ±0.14	5.46^{b} ±0.46
H-C+Pe+AA	4.12^{b} ±0.12	1.84^{ab} ±0.18	2.70^{a} ±0.02	0.58^{b} ±0.13	4.21^{ab} ±0.73

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

to that observed at 4 weeks (Table 3.18).

Table 3.19 shows the hepatic concentration of cholesterol in various groups of animals. Mice fed a pectin-rich diet for 4 weeks had liver cholesterol levels reduced by 40% of the control animals (control B). The hepatic concentrations of cholesterol in animals receiving ascorbic acid, however, remained unchanged. The simultaneous administration of pectin and ascorbic acid resulted in a reduction, but not significantly, of the hepatic cholesterol concentration. When pectin-rich diet was fed for 12 weeks, the hepatic concentration of cholesterol was further decreased (Table 3.20). On the other hand, the cholesterol level was increased by 28% following administration of ascorbic acid alone. When pectin and ascorbic acid were given concomitantly for 12 weeks, the hepatic level of cholesterol was found to be similar to those in animals given pectin alone (Table 3.20).

The histological examination of the liver sections showed that animals fed a 1% cholesterol diet with pectin plus ascorbic acid for 12 weeks, had no lipid accumulation (Plate 3.5). This finding is similar to that of the pectin-fed animals (Plate 3.3), but not to those receiving ascorbic acid alone (Plate 3.4).

TABLE 3.18 Effect of pectin and ascorbic acid either alone or in combination on plasma lipid profiles in mice fed a high cholesterol (1%) diet for 12 weeks.

Groups	T-C	TG	HDL-C (mmol/l)	LDL-C	HDL-C / LDL-C
H-C: (control B)	4.65^{a} ±0.32	1.49^{a} ±0.25	3.36^{a} ±0.20	0.63^{a} ±0.06	5.50^{a} ±0.34
H-C+Pe	2.87^{b} ±0.20	1.33^{a} ±0.11	2.07^{b} ±0.14	0.19^{b} ±0.04	12.21^{b} ±1.69
H-C+AA	3.32^{b} ±0.30	0.95^{a} ±0.10	2.29^{b} ±0.20	0.60^{a} ±0.08	4.00^{a} ±0.55
H-C+Pe+AA	2.63^{b} ±0.10	1.16^{a} ±0.26	1.92^{b} ±0.15	0.19^{b} ±0.05	12.50^{b} ±1.74

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.19 Effect of pectin and ascorbic acid either alone or in combination on the hepatic cholesterol concentration in high cholesterol (1%) fed mice for 4 weeks.

Groups	µmol/g liver	µmol/liver
H-C (control B)	62.2 ± 7.4^{a}	138.4 ± 28.1^{a}
H-C+Pe	39.1 ± 1.5^{b}	74.4 ± 6.9^{b}
H-C+AA	66.1 ± 6.8^{a}	144.0 ± 21.2^{a}
H-C+Pe+AA	47.4 ± 5.2^{ab}	94.2 ± 13.8^{ab}

Each value is the mean ± SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

TABLE 3.20 Effect of pectin and ascorbic acid either alone or in combination on the hepatic cholesterol concentration in high cholesterol (1%) fed mice for 12 weeks.

Groups	µmol/g liver	µmol/liver
H-C (control B)	84.9 ± 6.4^{a}	151.8 ± 14.4^{a}
H-C+Pe	42.9 ± 4.7^{b}	63.2 ± 8.2^{b}
H-C+AA	108.7 ± 4.8^{c}	202.7 ± 16.1^{c}
H-C+Pe+AA	38.9 ± 4.9^{b}	65.5 ± 9.9^{b}

Each value is the mean $\pm$ SEM of at least six animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

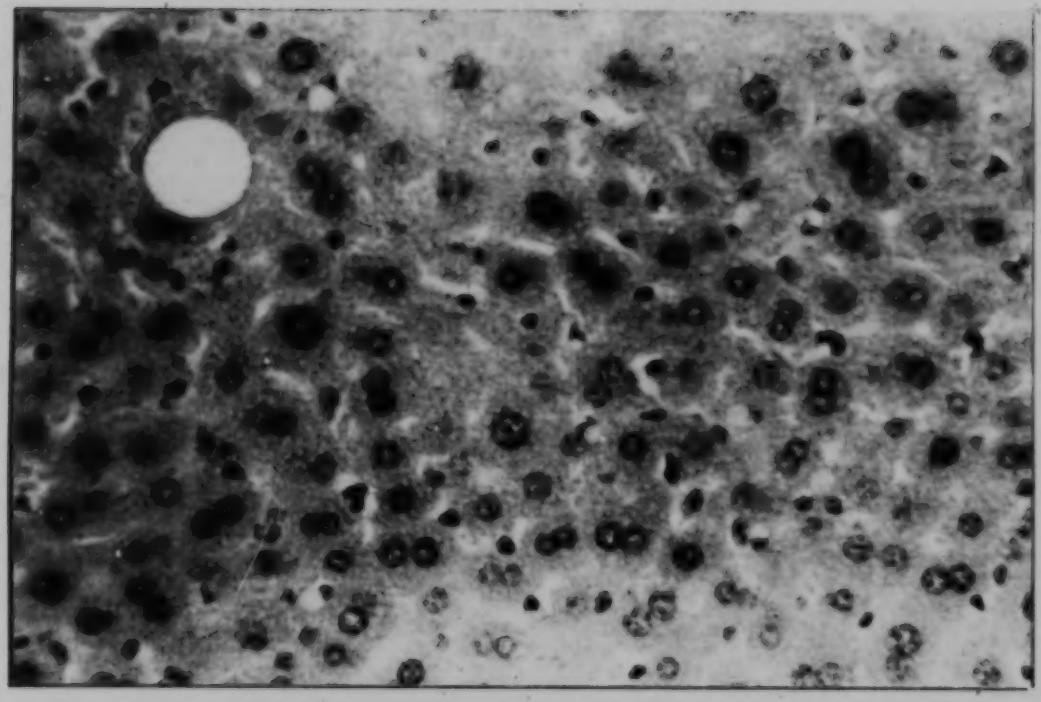

Plate 3.5 Photomicrograph of the liver from an animal fed a 1% cholesterol diet with pectin (5%) and ascorbic acid supplemented in drinking water (1 mg/ml) for 12 weeks (magnification 25X).

CHAPTER 4. DISCUSSION

The positive correlation between high levels of plasma cholesterol and CHD is well established (Consensus Conference, 1985 and Grundy, 1986). Epidemiological studies, animal experiments, and clinical research have shown large evidence linked dietary factors such as dietary cholesterol, total fat and saturated fatty acids, to the etiology of CHD (American Heart Association, 1986). It is known that lowering elevated plasma cholesterol levels reduces the risk of CHD. Thus, dietary modifications appear to be of essential importance in the reduction of hypercholesterolemia and prevention of CHD.

The present study investigated the short and long-term effects of two dietary factors, pectin and ascorbic acid either alone or in combination, on lipid status in mice fed a semi-synthetic diet containing 1% cholesterol. Most studies which have investigated the effects of dietary fiber and ascorbic acid on lipid metabolism have been carried out in rats and guinea pigs. As far as it is known, this is the first study carried out in mice to investigate the effects of the two dietary components on lipid status.

Mice are known to be a high resistant species to nutritionaly-induced hypercholesterolemia, controlling effectivelly the cholesterol homeostasis of the whole body by mechanisms involving supression of cholesterol synthesis, reduction of absorption and increased excretion of bile

acids (Corraze et al., 1985). In these animals, the hepatic cholesterol synthesis can be increased or decreased without altering the rate of LDL uptake; this compensatory capacity is much lower in man, which at basal state have a lower rate of cholesterol synthesis than that of rats and mice (Dietschy, 1985). Mice are therefore less suceptible to develop hypercholesterolemia when fed a high cholesterol diet. The use of a high cholesterol diet (1%) in this study is justified by the fact that the hypocholesterolemic effect of pectin appears to be most pronounced when animals are fed a diet supplemented with cholesterol (Vahouny and Cassidy, 1986). Furthermore, the hypolipidemic effect of ascorbic acid also appears to be more effective in hypercholesterolemic than in normocholesterolemic subjects (Ginter, 1975 and Ginter, 1979).

Results of the present study, however, indicated that feeding a diet containing 1% cholesterol for 4 or 12 weeks resulted in increased plasma and liver concentrations of cholesterol in ICR albino mice. The biochemical results are further supported by histological findings, thus the high cholesterol-fed animals had a greater lipid accumulation in their livers than those of the control group. These results are in close agreement with another study (Riley et al., 1980) which reported hypercholesterolemia in rats fed a diet containing the same amount of cholesterol (1%) and olive oil (10%) as in this study, for 4 and 6 weeks.

In mice the increases in plasma and liver lipids were

inhibited when the high cholesterol diet was supplemented with 5% pectin. The magnitude of inhibition was greater when the animals were fed the diet for 12 weeks than for 4 weeks. These findings were further substantiated by histological analysis showing that high cholesterol-induced hepatic accumulation of lipids was completely prevented by providing 5% pectin in the high cholesterol diet. This study also revealed that pectin administration altered did not only T-C levels but also the cholesterol concentration of LDL. Thus, feeding a high cholesterol diet containing 5% pectin for 4 and 12 weeks appeared to reduce plasma LDL-C concentrations by 40% and 70% respectively. These results are in agreement with others, who made similar observations in rats (Chen and Anderson, 1979; Judd and Truswell, 1985; Kritchevsky, 1987 and Vigne et al., 1987).

Elevated levels of HDL-C have been reported to be inversely related to the risk of CHD whereas LDL-C levels are directly related (Anderson and Tietyen-Clark, 1986). It was of interest that in the present study the animals fed a high cholesterol diet were found to be associated with raised plasma HDL-C and that the increase was inhibited when 5% pectin was added to the diet. These results are in agreement with many studies (Ahrens et al., 1986 and Kritchevsky, 1987), but contradictory to others (Chen and Anderson, 1979 and Chen and Anderson, 1981). It appears that most substances which lower plasma total cholesterol and LDL-C levels, also lower HDL-C concentration (Kritchevsky,

1987). It should be pointed out, however, that whereas the plasma HDL-C was reduced, the HDL-C/LDL-C ratio was significantly increased in animals receiving pectin for 12 weeks. Although the plasma HDL-C level appears to be reduced by dietary pectin, the significance of the increased HDL-C/LDL-C ratio in prediction of CHD cannot be ignored.

Supplementation of pectin (5%) to mice receiving a high cholesterol diet for 4 weeks reduced plasma triglyceride concentrations; however, this effect was not observed after 12 weeks of pectin feeding. The triglyceride lowering effect of pectin appear to be contradictory. Some studies (Anderson and Chen, 1979) failed to observe any effect of pectin on plasma triglyceride concentrations in rats, whereas other studies demonstrated significant reductions in plasma triglyceride levels in rats fed either a hypercholesterolemic or normocholesterolemic diet supplemented with pectin (1-10%) for approximately 4 weeks (Chen and Anderson, 1979, Chen et al., 1981, Vigne et al., 1987 and Ide and Horii, 1989). Thus, the triglyceride lowering effect of pectin given to high cholesterol-fed mice at 5% level for 4 weeks was confirmed in the present study.

The exact mechanism by which pectin lowers triglyceride concentration is still not clear. Studies undertaken to investigate the lymphatic absorption of lipids have indicated that pectin, may interfere with the digestion and/or absorption of emulsified triglycerides (Vahouny and Cassidy, 1986). Pectin may reduce lipid absorption by

causing intestinal morphofunctional changes, such as alteration of micellar solubility and reducing the lipid transport to epithelial cells (Vahouny et al., 1980 and Vahouny et al., 1988). The results of the present study do not, however, support the hypothesis that pectin could impair lipid absorption. Thus, pair-fed animals, receiving a 1% cholesterol diet supplemented with (5%) and without pectin for 8 weeks had similar fecal weights (dry and wet) and fecal cholesterol concentrations, indicating no effect of pectin on fecal bulk. The hypolipidemic effect of pectin was also observed in pair-fed mice receiving a 1% cholesterol diet supplemented with 5% pectin as evident by plasma TG and T-C concentrations. It is, therefore, unlikely that the lipid lowering effect of pectin is the reflection of any alteration in intake and/or absorption of dietary lipids. Furthermore, this study demonstrated that both pair and ad libitum fed animals receiving a high cholesterol diet supplemented with 5% pectin had the same weight gain compared to that of either control A or control B animals.

The lipid lowering effect of pectin has been related to its methoxyl content (Judd and Truswell, 1985). Low methoxyl pectin (less than 7% of methyl esters) has been reported to be less effective than high methoxyl pectin. It should be pointed out that in this study, low methoxyl pectin (6.1%) was used, and yet it was found to be significantly effective in reducing cholesterol levels in high cholesterol-fed mice.

It is probable that the observed cholesterol lowering

effect of pectin might be related to the sequestration of bile acids by pectin in the intestine (Kay and Truswell, 1977). The greater demand for bile acid synthesis due to increased bile acid elimination in the feces, may decrease the hepatic concentration of cholesterol. Thus, hepatic uptake of LDL might be increased, which in turn may lower the plasma total cholesterol and LDL-C concentrations (Mayes, 1988). Unfortunately, fecal bile acids were not measured in this study to substantiate this hypothesis.

In addition to pectin, ascorbic acid is another dietary factor which has been reported to have hypocholesterolemic effect (Ginter and Bobek, 1981, Ginter et al., 1982b, Horio et al., 1987). An experiment was designed to investigate the effect of this vitamin on lipid status in mice weighing 25-30 g. Ascorbic acid was given daily at a dose level of 1 mg/ml drinking water. The average water consumption of each animal was approximately 8 ml/day. Therefore, the total ascorbic acid intake per day per animal was about 8 mg. At this level of ascorbic acid intake, the hepatic concentrations of this vitamin were found to be similar between the supplemented and unsupplemented animals. Since mice can synthesize ascorbic acid, it could be argued that the supplemented amount of the vitamin was insufficient to exceed its physiological levels. One should point out however, that the supplemental dose level of approximately 8 mg/mouse weighing about 35 g is equivalent to more than 16 g of ascorbic acid for a 70 Kg human. This amount is at least

260 times of the RNI for vitamin C in humans. Ascorbic acid is a water soluble vitamin. Since this vitamin is not stored in tissues, its excess is normally excreted in urine. Unfortunately, urinary ascorbic acid concentrations were not measured in the present study.

Although ascorbic acid supplementation of 1 mg/ml drinking water failed to show any increase in the hepatic concentration of this vitamin, this dose level was found to be effective in lowering plasma TG concentration in mice. In the plasma, triglyceride output is mainly regulated by the enzyme lipoprotein lipase in extrahepatic tissues, such as adipose and muscle (Ginter et al., 1982a and Mayes, 1985). In the wall of blood capillary of the extrahepatic tissues, the triglycerides are hydrolyzed by the lipoprotein lipase to free fatty acids plus glycerol (Brown and Goldstein, 1984). An increased activity of the lipoprotein lipase has been reported in hypercholesterolemic animals on long-term ascorbic acid administration (Erden et al., 1985). It seems that ascorbic acid stimulates the lipolytic activity of the lipoprotein lipase, thus decreasing the plasma TG concentrations (Ginter et al., 1982a) as it was observed in the present study. However, the exact mechanism by which ascorbic acid stimulates the lipolysis of triglycerides is still not elucidated.

Ascorbic acid supplementation for 4 and 12 weeks, was also found to lower plasma T-C in high cholesterol fed animals. Nonetheless, the cholesterol lowering effect of

ascorbic acid was not observed on the hepatic cholesterol concentrations. The hepatic cholesterol concentration was, in effect, increased by 28% following ascorbate supplementation for 12 weeks. These findings were supported by the histological analysis which showed an excessive lipid accumulation in hepatocytes of the animals receiving a high cholesterol diet plus ascorbic acid. The underlying mechanism by which ascorbic acid produced fatty liver is not known. Hepatic storage of cholesterol is essentially in its ester form (Brown and Goldstein, 1984). Unfortunately in this study, hepatic cholesterol esters were not measured to better characterize the lipid deposition on liver samples.

It was of interest that the ascorbate-associated increase in hepatic cholesterol was in parallel with the high plasma levels of LDL-C but not with plasma T-C. It has been reported (Brown and Goldstein, 1984) that the synthesis of LDL-receptors is under a feedback control, thus increased cholesterol concentration in the liver cells leads to suppression of the LDL receptor activity, consequently increasing the plasma LDL-C levels.

Ascorbic acid is also known to interfere with the biosynthesis of bile acids through stimulating the activity of the enzyme 7α-hydroxylase, the rate limiting enzyme for cholesterol degradation (Ginter, 1975 and Ginter et al., 1982b) (see Fig 1.4). The ascorbic acid induced bile acid synthesis may lead to an increase in the pool of bile acids (Harris et at., 1979), which in turn, may result in a

decreased activity of the 7α-hydroxylation of cholesterol (a feedback inhibition), leading to hypercholesterolemia. In the present study, the elevated hepatic cholesterol levels observed in mice fed a high cholesterol diet supplemented with ascorbic acid may be related to this mechanism. Furthermore, studies carried out in hypercholesterolemic subjects receiving ascorbic acid supplementation (300-1000 mg/day) have revealed reductions in plasma cholesterol levels during the initial period of supplementation. After approximately 6 months of supplementation, however, the plasma cholesterol concentration tends to return to its pre-high levels (Ginter et al., 1981).

Ascorbic acid appears to stimulate the breakdown of cholesterol to bile acids, and pectin has the potential to eliminate the bile acids through binding these acids. It is therefore possible that the administration of ascorbic acid simultaneously with pectin may interrupt the enterohepatic reabsorption of bile acids, thus effectively lowering cholesterol status. Feeding pectin concomitantly with ascorbic acid to high cholesterol-fed mice for 4 and 12 weeks failed to support this proposed synergistic mechanism, in this study.

4.1. CONCLUSION

The work presented in this study has indicated that the supplementation of 5% pectin to mice fed a 1% cholesterol

diet was effective in lowering their lipid status, thus answering one of the questions proposed in this study. The long-term (12 weeks) feeding of pectin was found to be most effective in reducing hypercholesterolemia. The lipid lowering effect of pectin appeared not to be related to any alteration in dietary intake or absorption of lipids, since no differences were found in fecal weights and fecal cholesterol concentrations between the pair-fed animals receiving a high cholesterol diet with and without pectin for 4 weeks. The underlying mechanism by which pectin affects the plasma and liver lipid metabolism remains open for future investigations. It is possible that the hypocholesterolemic effect of pectin might be associated with the sequestration of bile acids in the intestine. Hence, the determination of fecal bile acids is an important suggestion for future work.

Ascorbic acid supplementation (1 mg/ml drinking water) appeared to be effective in lowering triglyceride status in mice. This observation is of interest since hypertriglyceridemia may also increase the risk for CHD (Grundy, 1984). However, this vitamin supplementation resulted in increased hepatic and decreased plasma cholesterol levels. The ascorbic acid-associated increase in hepatic concentration of cholesterol maybe the consequence of enterohepatic circulation of bile acids. This consequence was thought to be alleviated when pectin and ascorbic acid were administered concomitantly. A pilot study was carried

out to test this hypothesis in mice. The synergism between ascorbic acid and pectin in lowering cholesterol status in mice was not observed. The cholesterol-lowering effect observed in this study was essentially attributed to pectin.

Mice are able to synthesize ascorbic acid and to maintain a high level of this vitamin in their tissues even when they are fed an ascorbic acid-deficient diet for a long time (Ginter and Bobek, 1981). In the present study, ascorbic acid levels in the liver appeared to be unaffected by exogenous ascorbic acid at a dose level, 1 mg/ml drinking water/day for 4 weeks. It seems that although this level of ascorbic acid is exceedingly high in relation to human requirements, this was not high enough to exceed its physiological level in mice. Consequently the animals were unable to reduce cholesterol status. The efficacy of pectin in concomitant with ascorbic acid in lowering cholesterol status when given to species, which cannot synthesize ascorbic acid cannot be determined from the results of the present study. It is therefore suggested that a similar study is carried out in animals incapable of synthesizing ascorbic acid, such as guinea pigs.

REFERENCES

AHRENS, F., Hagemeister, H., Pjeuffer, M. and Barth, C.A. (1986). Effects of oral and intracecal pectin administration on blood lipids in minipigs. Journal of Nutrition, 116: 70-76.

ALLAIN, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P. (1974). The enzymatic determination of total serum cholesterol. Clinical Chemistry, 20: 470-475.

AMERICAN HEART ASSOCIATION (1986). Dietary guidelines for health american adults. Circulation, 74(6): 1465A-1468A.

ANDERSON, J.W. and Chen, W.J.L. (1979). Plant fiber carbohydrate and lipid metabolism. American Journal of Clinical Nutrition, 32: 346-363.

ANDERSON, J.W. and Tietyen-Clark, J.T. (1986). Dietary fiber: Hyperlipidemia, hypertension, and coronary heart disease. The American Journal of Gastroenterology, 81(10): 907-919.

BAIG, M.M. and Cerda, J. (1981). Pectin its interaction with serum lipoproteins. American Journal of Clinical Nutrition, 34: 50-53.

BASU, T.K. and Schorah, C.J. (1982). Vitamin C in Health and Disease. Chapters 5 and 8. The Avi Publishing Company, Inc, Connecticut; 152 p.

BATES, G.J., Mandal, A.R. and Cole, T.J. (1977). H.D.L. cholesterol and vitamin C status. Lancet, 2: 611.

BESSEY, O.A. (1950). Microchemical methods; Ascorbic acid. In: Vitamin Methods; Gyorgy, P. (ed). Academic Press, New York. Vol. 1, pp. 303-308.

BIERMAN, L.E. and Chait, A. (1988). Nutrition in relation to Hyperlipidemia and Atherosclerosis. In: Modern Nutrition in Health and Disease; Shils, M.E. and Young, V.R. (eds). Lea and Febiger, Philadelphia. pp. 1283-1296.

BJORKHEM, I. and Kallmer, A. (1976). Hepatic 7α-hydroxylation of cholesterol in ascorbate deficient and ascorbate-supplemented guinea pig. Journal of Lipid Research, 17: 360-365.

BROWN, M.S. and Goldstein, J.L. (1984). How LDL receptors influence cholesterol and atherosclerosis. Scientific American, 251(5): 58-66.

BURR, M.L., Battes, C.J., Milbank, J.E. and Yarnell, J.W.C. (1982). The relationship between plasma ascorbate and lipid concentrations in fasting men. Human Nutrition: Clinical Nutrition, 36 C: 135-139.

CANADIAN PHARMACEUTICAL ASSOCIATION (1987). Compendium of Pharmaceuticals and Specialties; Krosh, C., Gill, M., Hemmy, H., Oey, A., Gzywski, P. and Dumais. (eds).; Ottawa.

CHEN, W.J.L. and Anderson, J.W. (1979). Effect of plant fibre in decreasing plasma total cholesterol and increasing high density lipoprotein cholesterol. Proceedings of the Society for Experimental Biology and Medicine, 162: 310-313.

CHEN, W.J.L., Anderson, J.W. and Gould, M.R. (1981). Effects of oat bran, oat gum and pectin on lipid metabolism of cholesterol-fed rats. Nutrition Reports International, 24: 1093-1098.

CANADIAN CONSENSUS CONFERENCE ON CHOLESTEROL: A FINAL REPORT (1988). The canadian consensus conference on the prevention of heart and vascular disease by altering serum cholesterol and lipoprotein risk factors. Canadian Medical Association Journal, 139(11 suppl.): 1-8.

CONSENSUS CONFERENCE. (1985). Lowering blood cholesterol to prevent heart disease. JAMA, 253: 2080-2090.

CORRAZE, G.R., Lacombe, C.R. and Nibbelink, M.M. (1985). Dietary restrictions amplifies the hypercholesterolemic effect of cholesterol feeding in the rat. Nutrition Research, 5: 781-787.

DIETSCHY, J.M. (1985). Regulation of plasma low density lipoprotein cholesterol levels: The quantitative physiology of LDL turnover in animals and in man. Paper presented at the Japanese Conference on the Biochemistry of Lipids.

EINARSSOM, K. and Angelin, B. (1986). Hyperlipoproteinemia, Hypolipidemic Treatment and Gallstone Disease. In: Bile Acid and Atherosclerosis; Grundy, S.M. (ed). Vol. 15, pp. 67-97.

ERDEN, F., Gulenc, S., Torun, M., Kocer, Z., Simsek, B. and Nebioglu, S. (1985). Ascorbic acid effect on some lipid fractions in human beings. Acta Vitaminologica et Enzymologica, 7(1-2): 131-138.

FIDANZA, A., Audisio, and Mastroiacovo, P. (1982). Vitamin C and cholesterol. International Journal of Vitamin and Nutrition Research, [suppl] 23: 153-171.

FLETCHER, D.J. and Rogers, D.A. (1985). Diet and coronary heart disease: Helping patientes reduce serum cholesterol level. Postgraduate Medicine, 77(5): 319-328.

FOLCH, J., Lees, M. and Slone-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal sources. Journal of Biological Chemistry, 226: 497-509.

FRIEDWALD, W.T., Levy, R.I. and Fredickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparation ultra centrifuge. Clinical Chemistry, 18: 499-502.

GINTER, E. (1975). Ascorbic acid in cholesterol and bile acid metabolism. Annals of New York Academy of Sciences, 258: 410-421.

GINTER, E. (1979). Pretreatment serum cholesterol and response to ascorbic acid. Lancet, 2: 958-959.

GINTER, E., Kubek, F.J., Vozar, J. and Bobek, P. (1979). Natural hypocholesterolemic agent: Pectin plus ascorbic acid. International Journal of Vitamin and Nutrition Research, 49: 406-412.

GINTER, E. and Bobek, P. (1981). The influence of vitamin C on the lipid metabolism. In: Vitamin C; Cousell, J.N. and Horning, D.H. (eds). Applied Science Publishers, London. pp. 299-347.

GINTER, E., Bobek, P., Ozdin, L., Kubek, F.J. and Vozar, J. (1981). Synergism between ascorbic acid and bile acids binding agents. In: Hormones, Lipoproteins and Atherosclerosis. Advanced Physiology Science. Vol. 35, pp. 67-73.

GINTER, E., Bobek, P. and Jurcovicova, M. (1982a). Role of L-ascorbic acid in lipid metabolism. In: Ascorbic Acid: Chemistry, Metabolism and Uses; Seib, P.A. and Tolbert, B.M. (eds.). American Chemical Society, Washington, DC. pp. 381-393.

GINTER, E., Bobek, P., Kubek, F., Vozar, J. and Urbanova, D. (1982b). Vitamin C in the control of hypercholesterolemia in man. International Journal of Vitamin and Nutrition Research, [suppl], 23: 137-152.

GINTER, E. and Jurcovicova, M. (1987). Chronic vitamin C deficiency lowers fractional catabolic rate of low-density lipoproteins in guinea pigs. Annals of New York Academy of Sciences, 498: 473-475.

GOLDSTEIN, J.L. and Brown, M.S. (1984). Progress in understanding the LDL receptor and HGM-CoA reductase, two menbrane proteins that regulate the plasma cholesterol. Journal of Lipid Research, 25: 1450-1457.

GREGO, A.M. and LaRocca (1982). Correlation between chronic hypovitaminosis C in old age and plasma levels of cholesterol and triglycerides. International Journal of Vitamin and Nutrition Research [suppl], 23: 129-136.

GRUNDY, S.M. (1984). Hyperlipoproteinemia: Metabolic basis and rationale for therapy. American Journal of Cardiology, 54: 20c-26c.

GRUNDY, S.M. (1986). Cholesterol and coronary heart disease: A new era. JAMA, 256: 2849-2858.

GRUNDY, S.M. Vega, G.L. and Bilheimer, D.W. (1986). Causes and treatment of hypercholesterolemia. In: Bile Acids and Atherosclerosis; Grundy, S.M. (ed). Raven Press, New York. Vol. 15, pp. 13-39.

GRUNDY, S.M. (1987). Monounsaturated fatty acids, plasma cholesterol and coronary heart disease. American Journal of Clinical Nutrition, 45: 1168-1175.

GUYTON, A.C. (1986). Lipid Metabolism. In: Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia. pp. 818-827.

HARRIS, W.S., Kottke, B.A. and Subbianh, M.T.R. (1979). Bile acid metabolism in ascorbic acid-deficient guinea pig. American Journal of Clinical Nutrition, 32: 1837-1841.

HILLMAN, L.C., Peters, S.G., Ficher, C.A, and Pomare, E.W. (1985). The effects of the fiber components pectin, cellulose and lignin on serum cholesterol levels. American Journal of Clinical Nutrition, 42: 207-213.

HILLMAN, L.C., Peters, S.G., Ficher, C.A, and Pomare, E.W. (1986). Effect of the fiber components pectin, cellulose and lignin on bile salt metabolism and biliary lipid composition in man. Gut, 27: 29-36.

HOLLOWAY, D. and Rivers, J.M. (1984). Long-term effect of inadequate and excessive dietary ascorbate on bile acid metabolism in the guinea pig. Journal of Nutrition 114: 1370-1376.

HOOPER, P.L., Hooper, E.M., Hunt, W.C., Garry, P.J. and Goodwin, J. (1983). Vitamins, lipids and lipoproteins in a health elderly population. International Journal of Vitamin and Nutrition Research, 53: 412-419.

HORIO, F., Ozaki, K., Oda, H., Makino, s., Hayashi, Y. and Yoshida, A. (1987). Effect of dietary ascorbic acid, cholesterol and PCB on cholesterol concentrations in serum and liver in a rat mutant unable to synthesize ascorbic acid. Journal of Nutrition, 117: 1036-1044.

HORIO, F., Ozaki, K., Oda, H., Makino, s., Hayashi, Y. and Yoshida, A. (1989). Effect of dietary ascorbic acid, cholesterol and PCB on cholesterol and bile acid metabolism in a rat mutant unable to synthesize ascorbic acid. Journal of Nutrition, 119: 409-415.

HORSEY, J., Livesley, B., and Dickerson, J.W.T. (1981). Ischaemic heart disease and aged patients: Effect of ascorbic acid on lipoproteins. Journal of Human Nutrition, 35: 53-58.

IDE, T. and Horii, M. (1989). Hepatic carnitine and triglyceride lowering effect of dietary pectin in the rat. Nutrition Reports International, 39(4): 861-865.

ILLINGWORTH, D.R. and Sexton, G.J. (1984). Hypocholesterolemic effects of mevinolin in patients with heterozygous familial hypercholesterolemia. Journal of Clinical Investigation, 74: 1972-1978.

JACQUES, F.P., Hartz, S.C., Mctgandy, R.B. , Jacob . R.A. and Russel, R.M. (1987). Vitamin C and blood lipoproteins in an elderly population. Annals of New York Academy of Sciences, 498: 100-108.

JOHNSON, G.E. and Obenshain, S.S. (1981). Non responsiveness of serum high-density lipoprotein-cholesterol to high dose ascorbic acid administration in normal men. American Journal of Clinical Nutrition, 34: 2088-2091.

JUDD, P.A. and Truswell, A.S. (1982). Comparison of the effects of high and low methoxyl pectins on blood and fecal lipids in men. British Journal of Nutrition, 48: 451-458.

JUDD, P.A. and Truswell, A.S. (1985). The hypocholesterolemic effects of pectin in rats. British Journal of Nutrition, 53: 409-425.

KATTERMANN, R., Jawore, D. and Moller, G. (1984). Multicenter study of new enzymatic method of cholesterol determination. Journal of Clinical Chemistry and Clinical Biochemistry, 22: 245-251.

KAY, R.M. and Truswell, A.S. (1977). Effect of citrus pectin of blood lipid and fecal sterol excretion in man. American Journal of Clinical Nutrition, 30: 171-175.

KAY, R.M. (1986). Dietary fiber - A perspective. In: CRC Handbook of Dietary Fiber in Human Nutrition; Spiller, G.A. (ed). CRC Press, Florida. pp. 7-11.

KASANIEMI, Y.A. and Grundy, S.M. (1984). Influence of probucol on cholesterol and lipoprotein metabolism in man. Journal of Lipid Research, 25: 780-790.

KONO, K., Hayakawa, M., Asai, K. and kuzuya, F. (1988). Cholesterol metabolism in inherently scorbutic rats. Journal of Nutrition Science and Vitaminology, 34: 35-45.

KRITCHEVSKY, D. (1987). Dietary fiber and lipid metabolism. International Journal of Obesity, 11 [suppl] (1): 33-43.

LEE, J.J. and Han, I.K. (1985). Effect of dietary pectin levels and kinds of lipids on serum and tissues cholesterol levels in rats. Korean Journal of Animal Science, 27(11): 724-727.

LINSCHEER, W.G. and Vergroesen, A.J. (1988). Lipids. In: Modern Nutrition in Health and Disease; Shils, M.E. and Young, V.R. (eds). Lea and Febiger, Philadelphia. pp. 72-107.

LIPID RESEARCH CLINICS PROGRAM: The lipid research clinics coronary primary prevention trial results: I. Reduction in incidence of coronary heart disease. (1984). JAMA, 251: 351-364.

LOPES-VIRELLA, M.F. Stone, P., Ellis, S. and Colwell, J.A. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. Clinical Chemistry, 23: 882-884.

MAYES, P.A. (1985). Metabolism of Lipids: II. Role of the tissues. In: Harper's Review of Biochemistry; Martin, D.w., Mayes, P.A. , Rodwell, V.W. and Granner, D.K. (eds). Lange Medical Publications, California. pp. 249-256.

MAYES, P.A. (1988). Cholesterol Synthesis, Transport and Excretion. In: Harper's Biochemistry; Murray, R., Granner, D.K., Mayes, P.A. and Rodwell, V.W. (eds). Appleton and Lange, California. pp. 241-252.

McGILVERY, R.W. (1983). Turnover of fats and lipoproteins: the cholesterol conection. In: Biochemistry: A functional approach; McGilvery, R.W. and Goldstein, G.W. (eds). pp. 555-571.

McMURRAY, W.C. (1983). Lipids. In: Essentials of Human Metabolism. Harper and Row, publishers, Philadelphia, Pensylvania. pp. 160-205.

MIETTINEN, T.A. and Kasaniemi, Y.A. (1986). Cholesterol balance and lipoprotein metabolism in man. In: Bile Acid and Atherosclerosis. Grundy S.M. (ed). Raven Press, New York. Vol. 15, pp. 113-155.

O'BRIEN, B.C. and McMurray, D.N. (1988). Human plasma lipid and immunologic responses to eggs and ascorbic acid. Nutrition Research, 8: 353-366.

PEARSON, S., Stern, S. and McGavack, T.H. (1953). A rapid, accurate method for determination of total cholesterol in serum. Analytical Chemistry, 25: 813-814.

PETERSON, V.E., Craps, P.A., Weininger, J., Ginsbers, H. and Olefsky, J. (1975). Quantification of plasma cholesterol and triglyceride levels in hypercholesterolemic subjects receiving ascorbic acid supplements. American Journal of Clinical Nutrition, 28: 584-587.

PYORALA, K. (1987). Dietary cholesterol in relation to plasma cholesterol and coronary heart disease. American Journal of Clinical Nutrition, 45: 1176-1184.

RIFAI, N. (1986). Lipoproteins and apolipoproteins: composition, metabolism and association with coronary heart disease. Archives of Pathology and Laboratory Medicine, 110: 694-701.

RIFKIND, B.M. (1986). Diet, plasma cholesterol and coronary heart disease. Journal of Nutrition, 116: 1578-1580.

RILEY, J.W., Glickma, R.M., Green, P.H.R. and Tall, A.R. (1980). The effect of chronic cholesterol feeding on intestinal lipoproteins in rat. Journal of Lipid Research, 21: 942-952.

ROE, J.H. and Keuther, C.A. (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivation of dehydroascorbic acid. Journal of Biological Chemistry, 147: 399-407.

SABLE-AMPLIS, R., Sicart, R. and Dupouy, D. (1987). Hepatic cholesterol 7α -hydroxylase activity in hamsters fed diets enhiched with pection and/or cholesterol. Annals of Nutrition Metabolism, 31: 61-68.

SCHNEEMAN, B.O., Cimmarusti, J., Cohen, W., Downes, Z. and Lefevre, M. (1984). Composition of high density lipoproteins in rats fed various dietary fibers. Journal of Nutrition, 114: 1320-1326.

SHINNICK, F.L., Longacre, M.J., Ink, S.L. and Marlett, J.A. (1988). Oat fiber: composition versus physiological function in rats. Journal of Nutrition, 118: 144-151.

SOUTHGATE, D.A.T., Spiller, G.A., White, M. and McPherson-Kay, R.M. (1986). Glossary of dietary fiber components. In: CRC Handbook of Dietary Fiber in Human Nutrition; Spiller, G.A. (ed). CRC Press, Florida. pp. 29-33.

STRYER, L. (1981). Biosynthesis of membrane lipids and steroid hormones. In: Biochemistry. W.H. Freeman and Company. pp. 470-473.

SUPERKO, H.R., Haskele, K.W., Sawrey-kubicek, L. and Farquhar, W.J. (1988). Effects of solid and liquid guar gum on plasma cholesterol and triglyceride concentrations in moderate hypercholesterolemia. American Journal of Cardiology, 62: 51-55.

VAHOUNY, G.V., Roy, T., Gallo, L.L., Story, J.A., Kritchevsky, D. and Cassidy, M.M. (1980). Dietary Fibers III. Effects of chronic intake on cholesterol absorption and metabolism in the rat. American Journal of Clinical Nutrition, 33: 2182-2191.

VAHOUNY, G.V. and Cassidy, M.M. (1986). Effect of dietary fiber on intestinal absorption of lipids. In: CRC Handbook of Dietary Fiber in Human Nutrition ; Spiller, G.A. (ed). CRC Press, Florida. pp. 121-128.

VAHOUNY, G.V., Stachithanandam, S., Chen, J., Tepper, S., Kritchevsky, D., Lightfoot, F. and Cassidy, M.M. (1988). Dietary fiber and intestinal adaptation: Effects on lipid absorption and lymphatic transport in the rat. American Journal of Clinical Nutrition, 47: 201-206.

VEGA, G.L. and Grundy, S.M. (1987). Mechanism of primary hypercholesterolemia in humans. American Heart Journal, 113: 493-502.

VIGNE, J.L., Larion, D., Bores, P., Portugal, H., Pauli, A.M., Hauton, J.C. and Lafant, H. (1987). Effect of pectin, wheat bran and cellulose on serum lipids and lipoproteins in rats fed a low or high-fat diet. British Journal of Nutrition, 58: 405-413.

WAHLEFELD, A.W. (1974). Triglycerides. Determination after enzymatic hydrolysis. In: Methods of Enzymatic Analysis; Bergmeyer, H.U. (ed). Press, Inc, New York and London. Vol. 4, pp. 1831-1835.

WATSON, D. (1960). A simple method for the determination of serum cholesterol. Clinical Chimica Acta, 5: 637-643.

WINER, B. (1971). Statistical principles in experimental design. Chapter 3. McGraw-Hill Book Company, New York.

WOLTHUIS, M.S., Albers, H.F. Jeveren, J.C., Jong, J.W., Hautvast, J.J., Hermus, R.J., Katan, M.B., Brydon, W.G. and Eastwood, M.A. (1980). Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids and colonic function. American Journal of Clinical Nutrition, 33: 1745-1756.